MOTOR UNIT PROPERTIES FOLLOWING CROSS-REINNERVATION OF CAT TRICEPS SURAE MUSCLES.

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1985

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee, Drs. John Munson, Don Stehouwer, George Sypert and Floyd Thompson, for their advice and interest throughout the course of my dissertation project. Dr. Janet Zengel was extremely helpful at several stages of this project. Expert technical assistance was provided by William Ireland, C.J. Thomas and Audrey Kalehua.

I am especially grateful to Drs. John Munson and George Sypert, who have been excellent teachers, admirable role models, and great company for the past few years.

Deb and Christen deserve special thanks for putting up with the life of a graduate student, and making this all seem worthwhile.

Finally, I thank all those responsible for the microcomputer.

I have been supported as a graduate research associate on an National Institute for Neurological and Communicative Diseases and Stroke (NINCDS) grant to Dr. John Munson. The W.L. Gore corporation donated Gore-Tex for use as nerve-sleeves.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MOTOR UNIT PROPERTIES FOLLOWING CROSS-REINNERVATION OF CAT TRICEPS SURAE MUSCLES

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August, 1985

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Major Department: Neuroscience

This study showed several aspects of the relationships between cat triceps surae alpha-motoneurons, and the muscle fibers they innervate. Relationships between motoneuron electrical properties and muscle unit contractile properties were investigated for normal medial gastrocnemius (MG), lateral gastrocnemius (LG) and soleus motor units. The relationships between motoneuron and muscle fibers were similar in quality and strength for normal LG and soleus and for normal MG. Surgical section of the MG nerve, followed by re-anastomosis to MG nerve (self-reinnervation), or to the combined LG-soleus nerve (cross-reinnervation), was employed to examine motoneuron influence on muscle phenotype, and effects of muscle on the expression of motoneuron electrical properties. Functional connection to muscle was a necessary condition for expression of normal, mature motoneuron electrical properties. Long-term self-reinnervation resulted in complete recovery of motoneuron electrical properties, muscle unit contractile properties, and the relationships between them. Reinnervation of a foreign muscle (LG), with similar original muscle fiber type composition to MG, also resulted in complete recovery of these parameters. Muscle fibers

of soleus, an almost purely slow muscle, resisted the influence of MG motoneurons. Since MG motoneurons failed to convert soleus muscle fibers, MG motoneuron properties were altered, thus maintaining a close relationship between motoneuron electrical type and muscle unit contractile type. This relationship was of similar strength to that between normal and also self-reinnervated MG motoneurons and MG muscle fibers, but of different quality . In MG motoneurons which innervated soleus muscle, incomplete recovery from axotomy is a possible explanation for the altered values for motoneuron rheobase, input resistance and axonal conduction velocity. Afterhyperpolarization potential (AHP) half-decay time of MG motoneurons which innervated soleus muscle was longer than in MG motoneurons which innervated MG muscle, whereas axotomy resulted in no change in mean AHP half-decay time. Incomplete recovery cannot explain this finding. Thus type of muscle innervated influences expression of motoneuron electrical properties. A retrogradely transported chemical message from muscle is a likely mediating mechanism.

CHAPTER I
INTRODUCTION

The mammalian neuromuscular system has been an important model system for study of how cells influence the expression of properties of other cells. In particular, the expression of muscle phenotype has been shown to be under strong neural influence. Buller, Eccles, and Eccles surgically re-routed the nerve from the 'slow' soleus muscle into the 'fast' flexor hallucis longus muscle (FHL), and *vice versa*, in young cats (cross-reinnervation; Buller *et al.* 1960). This study illustrated two major points. First, the type of nerve influenced the contractile speed of the whole muscle; the 'fast' FHL muscle became slow under soleus nerve influence, and the 'slow' soleus muscle became faster, when innervated by FHL nerve. Second, the conversion of 'fast' muscle by a 'slow' nerve was more complete than the reverse. These initial observations have been repeated several times, and at finer levels of resolution, including single motor unit studies (see references cited in CHAPTER III). Collectively, these studies provide strong evidence for neural regulation of muscle phenotype.

The electrical activity pattern of cross-reinnervated muscles is that of the muscle originally innervated by the nerve (Sperry, 1945; Cohen, 1978; Brinkman *et al.* 1983; Mulkey, 1983; O'Donovan *et al.*, in press). That is, the activity pattern of the nerve is unchanged when innervating a foreign muscle. This observation has led most workers to

regard motoneuron properties as uninfluenced by the particular muscle innervated (e.g. Gordon, 1983). A similar conclusion was reached by Kuno et al. (1974b), who measured action potential overshoot, axonal conduction velocity, and afterhyperpolarization (AHP) duration in soleus motoneurons, up to five months after self- or cross-reinnervation of flexor digitorum longus muscle (FDL).

Relatively little is known, however, about the expression of motoneuron electrical properties, and the relationships between them, after reinnervation of a foreign muscle. A complication in early studies was that motor units within a muscle were regarded as a single, homogeneous population. Thus Kuno et al. regarded medial gastrocnemius (MG) or FDL motoneurons as uniformly 'fast', and soleus motoneurons as uniformly 'slow'. MG and FDL, although predominately 'fast', are actually 'mixed' muscles, containing several types of motor unit, and soleus has occasional fast units (reviewed in Burke, 1981). Changes may be differential between motor unit types, following reinnervation.

This study utilizes the the cat triceps surae neuromuscular system as an experimental model of motor unit properties. The triceps surae includes the soleus, MG and lateral gastrocnemius muscles (LG), all of which are ankle extensors. The motor unit is defined as an alpha motoneuron and the muscle fibers it innervates, the muscle portion alone being referred to as the muscle unit (Burke, 1981). MG motor units can be classified into four types, based on contractile characteristics of the muscle unit (Burke, 1981). Motor units can be divided into fast or slow on the basis of twitch time-to-peak, or the tension profile in an unfused tetanus ('sag'; Burke et al. 1973). The fast units can be

subdivided on the basis of resistance to fatigue, into fast-fatiguable (type FF), fast-fatigue-resistant (FR), and fast with intermediate fatigue-resistance (FI). All slow, type S units are highly fatigue-resistant. Normal MG contains approximately 45% type FF, 5% type FI, 25% type FR and 25% type S motor units (Burke, 1981; Fleshman et al. 1981). Normal soleus contains nearly 100% type S units (Burke, 1981). Normal LG has not previously been studied independent from MG, but is considered to be similar to MG in motor unit types (from muscle histochemistry; Ariano et al. 1973; see also Hamant, 1977).

Motoneuron electrical properties have been shown to vary according to motor unit type, in MG (Fleshman et al. 1981; Zengel et al. 1985). Parameters investigated include axonal conduction velocity, AHP half-decay time (related to the ability of the cell to fire repetitively), rheobase (an inverse indicator of cell excitability), and input resistance (influenced by cell size and geometry, as well as specific membrane conductances). Zengel et al. (1985) devised a method to classify motoneurons on the basis of their electrical characteristics. This scheme was found to predict motor unit type, determined independently by contractile properties, with greater than 90% accuracy. In light of the close relationship evident between motoneuron electrical properties and muscle unit contractile properties in normal MG, it is of interest whether this is a general property of normal motor units of other triceps surae muscles, and whether motoneurons exhibit plasticity in response to experimental manipulation.

In this study, experimental alteration of the innervation of triceps surae muscles is used to investigate several aspects of the

relationship between motoneuron and muscle. MG motoneurons were isolated by intracellular methods, to allow recording of motoneuron electrical properties. Intracellular current injection was employed to activate the muscle unit to measure contractile responses. In addition, whole muscle contractile properties and muscle histochemical properties were documented. Acute experiments were performed on normal, unoperated-on animals, animals whose MG nerve had been sectioned (prior to reinnervation of muscle), and animals whose MG and LG-soleus (combined nerve) nerves had been sectioned, and allowed to reinnervate either the original muscle (self-reinnervation), or the opposite muscle(s) (cross-reinnervation). Acute experiments for reinnervated muscles were performed at various times after the initial surgery (from three weeks to ten months). All studies herein that involved surgical manipulation, were analyzed for MG motoneurons only.

The questions addressed in this study are as follows:

1) Are the relationships between motoneuron electrical properties and muscle unit contractile properties of similar quality and strength in LG and soleus to that observed in MG, (CHAPTER V)?

2) Is functional connection to muscle required for expression of normal, mature motoneuron electrical properties (CHAPTER IV)?

3) Do motoneuron electrical properties recover to control values upon reinnervation of the original muscle (CHAPTER III)?

4) What is the time course and pattern of recovery during self-reinnervation (CHAPTER IV)?

5) Given a 'choice' of end organs, is there selectivity of reinnervation on the basis of motor unit type (CHAPTER VI)?

6) What are the limits of motoneuron influence upon muscle properties (CHAPTER VI)?

7) Does the expression of motoneuron electrical properties depend upon the particular muscle innervated; i.e. is there a regulatory influence of muscle, upon motoneurons (CHAPTER VII)?

CHAPTER II
METHODS

Experiments were performed on 51 adult cats (22 normal cats, 29 experimental). Animals were anesthetized to a depth sufficient to maintain areflexia, during all surgical and experimental procedures. All animals were adult at the time of the initial surgery.

Initial Surgery

Animals were anesthetized with a gaseous mixture of oxygen, nitrous oxide, and halothane. Under aseptic conditions, the MG and LG-S nerves of the left hind leg were surgically isolated within the popliteal fossa, sectioned about 15-25mm proximal to the triceps surae muscles, and the proximal stumps of each nerve were resutured to the distal stump leading to the muscle(s) not innervated by that nerve originally (X-reinnervation; Fig. 2-1) or to the stump leading to the original muscle (self-reinnervation). In some experiments this procedure was also carried out on the right leg. To direct axonal growth, 15mm long sleeves of Gore-Tex (30um internodal distance) were used (Young et al. 1984; see their Fig. 2). The two nerve ends were joined and their orientation fixed with two 9/0 sutures through the epineurium.

Following recovery animals were maintained in pairs in 90x90cm cages. The animals received care and were exercised daily outside of the cage.

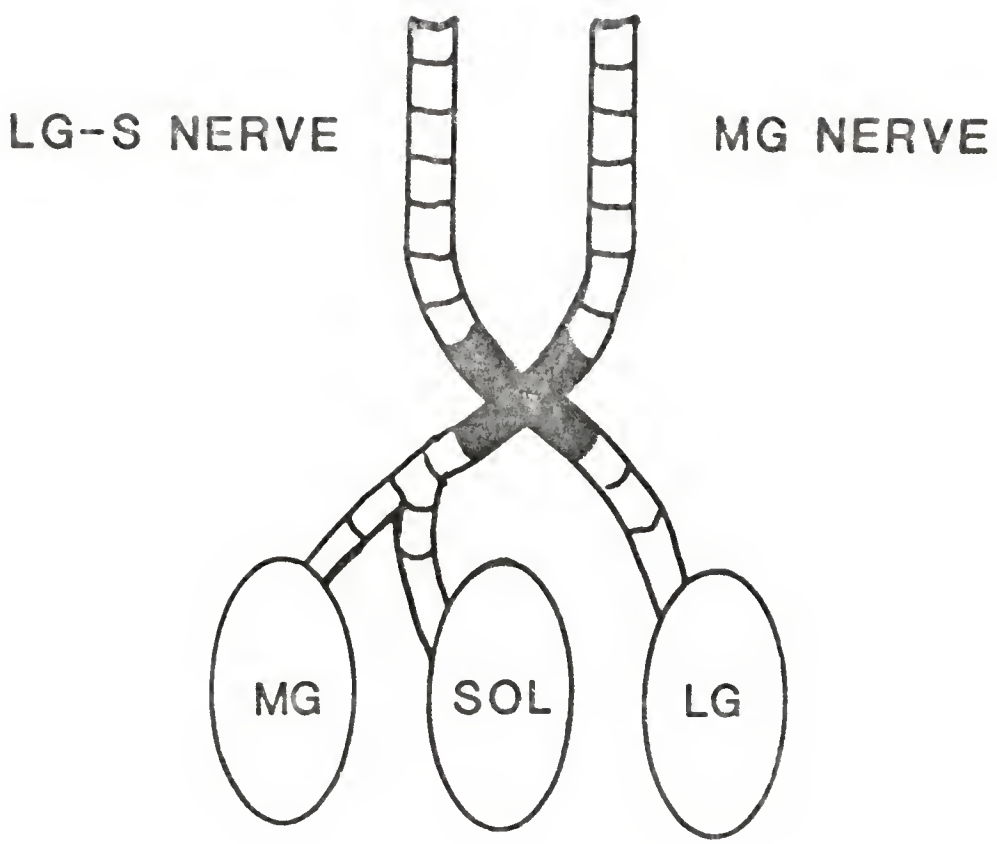

Figure 2-1. Schematic diagram of cross-reinnervation surgery. Location of Gore-Tex sleeves shown as black cylinders.

Acute Experiments

The acute experiments utilized techniques and protocol published previously (Fleshman *et al.* 1981; Zengel *et al.* 1985). Operated animals were studied three weeks to 11 months following the initial surgery. Animals were anesthetized via intraperitoneal injection of sodium pentobarbitol (35mg/kg). Leg, body, and spinal cord temperatures were maintained at 35°-37°C with a heat lamp and heating pad.

The unoperated, normal MG population derives from 16 animals (15 females, one male; 2.0 to 6.4 kg, mean 3.5 kg). The normal LG and soleus data derive from 15 animals (eight animals were the same as for MG; 14 females and one male; 1.9 to 3.8 kg, mean 2.8kg).

Four acute experiments were performed on animals (all females, 2.8 to 3.4 kg, mean 3.0 kg) whose MG nerve was sectioned and had not yet reinnervated muscle (*no-re*; 20-35 days post-operative). Eleven animals were examined at various times after self-reinnervation of MG muscle. Two animals (both female, 3.0 and 3.3 kg) were investigated five to six weeks following the initial surgery (*low-re*; these animals had whole muscle twitch tensions of less than 200g-wt). Two additional animals (both female, 2.9 and 3.3 kg) were examined nine to ten weeks post-operative (*med-re*). These animals had whole muscle twitch tensions approximately 50% of long self-reinnervated MG. Five animals (four female, one male, 2.9 to 3.6 kg, mean 3.4 kg) were examined nine months after self-anastomosis of the MG nerve (*long-re*). A final two animals (both female, 3.5 and 2.9 kg) were examined whose recovery was intermediate between the low-re and med-re stages .

Cross-anastomosis of the MG and LG-soleus nerves was accomplished for 14 animals (all females). Four of these were investigated at nine-to-ten weeks (medX, 2.0 to 2.8 kg, mean 2.4 kg). The remaining ten animals were examined nine-to-11 months post-operatively (longX, 2.2 to 3.7 kg, mean 2.9 kg).

Whole Muscle Twitch

Prior to single unit recording, whole muscle twitch contractions were obtained by stimulating the MG or LG-S nerve, with the MG, LG, or soleus tendon attached to a force transducer. Each muscle was tested in response to each of the two nerves, against a passive tension of 100g-wt., and also in response to the nerve eliciting the greater tension, at the length providing maximal tension. The muscles were activated tetanically before each measurement to potentiate the twitch response. In some animals whole muscle twitches were obtained again following single unit studies. The MG and LG muscle were then completely separated and the series of twitches recorded again.

Motor Unit Studies

MG motoneurons were identified by antidromic stimulation of the MG nerve, central to the nerve sleeve (innervates MG in self-reinnervation, LG and soleus in cross-reinnervation). Motoneurons were impaled with 3M KCl-filled microelectrodes, with impedances in the 4-12 Mohm range (measured at 1KHz). The muscle innervated by the MG motoneuron, was differentiated by intracellular current injection, and locating the muscle response.

Methods for obtaining and analyzing motoneuron and muscle unit data were as reported previously (Fleshman et al. 1981; Zengel et al. 1985).

We recorded antidromic action potential conduction time, rheobase, input resistance [by the direct method of measuring voltage deflection by means of a bridge circuit while injecting a 1nA current, without correction for nonlinearities, (Ito and Oshima, 1965)], and after-hyperpolarization potential (AHP) for each cell. The derived value rheobase/input resistance was calculated for each motor unit because that value (in conjunction with AHP) has been shown to precisely categorize the unit type to which a motoneuron belongs in normal MG (Zengel et al. 1985). The time for the AHP to decay to half-maximum amplitude (AHP half-decay time) was measured. Only motoneurons with action potential amplitude of 60mV, or greater, were used for analysis of motoneuron electrical properties. Muscle unit contractile data were obtained from a few units where motoneuron action potentials were less than 60mV.

The contractile responses of motor units were activated by intracellular current injection at the motoneuron, and measured using the methods of Burke et al. (1973). Muscle unit contractile responses were measured against a passive tension of 100g-wt. The following responses were recorded: unpotentiated twitch; potentiated twitch (following 100Hz tetanic stimulation); unfused tetanus ('sag'); tetanic tension (600 ms at 100 Hz for fast units, 1500 ms at 100Hz for slow units) and the fatigue test (Burke et al. 1973).

Motor units were classified on the basis of potentiated twitch time-to-peak (fast units had a time-to-peak of potentiated twitch of 40ms or less and exhibited sag, slow units had time-to-peak of > 40ms and did not sag) and fatiguability (FF units had fatigue index of <

0.25, S and FR units had fatigue indices of > 0.75, and FI units were in between) as type FF, FI, FR, or S (Burke et al. 1973; Fleshman et al. 1981). Units with twitch time-to-peak in the range 35-45ms were classified as fast or slow by the presence (fast) or absence (slow) of "sag" (Burke et al. 1973) in an unfused tetanus (inter-stimulus interval = 1.25X time-to-peak).

Muscle Histochemistry

Animals were sacrificed by overdose of sodium pentobarbitol. The left MG, LG, and soleus muscles were excised, cleaned of excess connective tissue, blotted dry, and weighed to the nearest 0.1g. For MG and soleus, a 10mm block of tissue was removed from the thickest part of the muscle belly, cutting perpendicular to muscle fiber direction. Lateral gastrocnemius muscles were cut into three blocks: proximal, middle, and distal, each including approximately one third of the muscle length. The blocks of muscle were fixed to a piece of cork with gum tragacanth. Muscle orientation was carefully noted. The cork and muscle were then immersed in isopentane cooled to -160°C by immersion in liquid nitrogen. The tissue was then placed in a cryostat maintained at -20°C. After a few minutes drying time, the frozen tissue was wrapped in parafilm, placed in a plastic vial, and stored until cut and stained.

Muscle histochemistry was performed within one week of the acute experiment. The tissue and cork were mounted on a cryostat chuck with OCT compound. Ten um thick serial sections were cut from each block, and stained for myosin ATPase (preincubated at pH 10.3 and 4.2,; Padykula and Herman, 1955; Guth and Samaha, 1970), Nicotinamide adenine

dinucleotide diaphorase (NADH-D; Novikoff et al. 1961) and α-glycerophosphate dehydrogenase (GPD; Wattenberg and Leong, 1960).

Muscle fiber types were classified using the system of Peter et al. (1972). This scheme designates fibers as fast or slow, on the basis of alkaline myosin ATPase (dark= fast; light = slow). Fast fibers are further subdivided into fast glycolytic fibers (FG), staining light for NADH-D and dark for GPD, and fast oxidative glycolytic (FOG), staining dark for both NADH-D and GPD. Slow (SO) fibers stain dark for NADH-D and light for GPD.

The distribution of muscle fiber types was determined from projections of the original slides with a microprojector. For LG, approximately 600-1000 fibers were analyzed for myosin ATPase and NADH-D, from each of the four compartments of the muscle (English and Letbetter, 1982a,b). The LGm compartment was analyzed in the most medial portion of the proximal section (separated by tendon). The LG2 compartment was located just lateral to the LGm compartment, in the middle section. LG1 was analyzed from the distal section, near the lateral border (lateral to tendonous inscription). Finally, LG3 was located near the medial border of the distal section (see English and Letbetter, 1982b; their Fig. 3). For soleus, the number of fibers with "fast" (type II; Engel, 1970) staining characteristics were counted. For MG approximately 1000 fibers from each of three separate areas (single cross-section) were sampled. Muscle fiber areas were determined by planimetry from photographs for 25-50 fibers of each type (FG,FOG,SO) for each compartment of LG and for each MG or soleus muscle.

Following single unit studies in two long self-reinnervation experiments in which both MG nerves had been sectioned and re-anastomosed, a single type FF motor unit was isolated on the contralateral side of the animal by intracellular impalement. After measurement of motor unit properties, the unit was repetitively activated using the same stimulus regime as for the fatigue test. This was repeated over approximately one half hour, with one minute rest between each two minute stimulation period, in order to deplete the muscle fibers of that motor unit of their stores of glycogen (Edstrom and Kugleberg, 1968; Kugleberg et al. 1970). These muscles were prepared as above for muscle histochemistry. An additional serial section was stained for glycogen (PAS stain). The muscle was sectioned until the section with the maximum number of depleted fibers was located. Serial sections for myosin ATPase and NADH-D were examined to determine whether reinnervated motor units were homogeneous with respect to muscle fiber types and to examine their distribution.

Statistical Considerations

All statistical analysis was performed with programs written in the language of the Statistical Analysis System (SAS; Helwig and Council, 1979). Mann-Whitney U-test was used to determine differences between two means. Wilcoxon's multiple range test was used to compare multiple means. Post-hoc analysis with Tukey's range test was used to determine which specific means were different. Significant differences between the proportions of motor unit or muscle fiber types were determined by the chi square test, and comparison to the hypergeometric distribution. An alpha value of 0.01 was considered to be significant as different

control samples ocassionally differed at the 0.05 level. SAS programs were executed with the facilities of the Northeast Regional Data Center at the University of Florida.

CHAPTER 3
NORMAL MG AND LONG-TERM SELF-REINNERVATION OF MG

Introduction

Since the initial cross-reinnervation study of Buller et al. (1960), attention has been focused on the role of the motoneuron in dictating muscle properties. Numerous studies of contractile properties at the whole muscle (Close, 1965; Close and Hoh, 1969; Luff, 1975; Prewitt and Salafsky, 1967) and single unit levels (Bagust et al. 1981; Burke, 1980; Burke et al. 1979; Chan et al. 1982; Dum et al. 1979; Lewis et al. 1982) have supported the original hypothesis of neural determination of muscle properties, and extended its documentation to finer levels of organization.

In addition, experiments in which muscles of mixed fiber type composition were subjected to chronic electrical stimulation indicate that the frequency and/or amount of muscle activity are important factors in the determination of muscle contractile properties (Eerbeek et al. 1984; Goldring et al. 1981; Hudlicka et al. 1982; Lomo et al. 1974, 1980; Salmons and Vrbova, 1969; Smith, 1978; see additional references in Pette, 1984; Salmons and Henrikson, 1981).

All of these studies (cross-reinnervation and stimulation) indicate that it is easier to change a fast muscle to slow than the reverse. The chronic stimulation experiments suggest that neural activity plays a

major role in determining muscle fiber characteristics, but there appear to be limits to the extent of this influence.

Only two laboratories have previously related motor unit properties to motor unit type following reinnervation. The motoneuron properties investigated were axonal conduction velocity (Burke, 1980; Gordon and Stein, 1982a,b), AHP duration (Burke, 1980), and extracellular action potential amplitude (Gordon and Stein, 1982a,b). All studies reported recovery to control levels by nine months to one year post-operative.

Kuno et al. (1974b) reported that five months following the initial surgery, conduction velocity, resting membrane potential, AHP duration, and action potential overshoot were incompletely recovered in reinnervated cat MG and soleus motoneurons. In that study all MG motoneurons were considered 'fast' and soleus motoneurons were considered 'slow. Czeh et al. (1978) suggested that AHP duration is regulated by retrograde trophic messages from muscle. There is some indication of a muscle influence on axonal conduction velocity as well (Burke, 1980; Lewis et al. 1978). Thus, in addition to neural induction of muscle properties there may also be retrograde influence upon the motoneuron from the muscle.

In light of the differences in normal animals between motor unit types in their motoneuron electrical and muscle unit contractile properties (c.f. Fleshman et al. 1981; Zengel et al. 1985), it is of interest whether motoneuron electrical properties, muscle unit contractile properties, and their interrelationships, are restored following self-reinnervation. The difference between slow soleus and the overall MG motoneuron sample in the studies of Kuno and colleagues

(Kuno, 1984) suggests that fast and slow motor units in a mixed muscle may show different responses to axotomy and subsequent reinnervation.

A second question in reinnervation of adult skeletal muscle is whether all motoneurons have an equal ability to reinnervate and maintain connections with muscle fibers. Lewis et al. (1982) observed a dramatically increased amount of type I (associated with slow motor units) muscle fibers in one cat flexor hallucis longus (FHL) muscle three years following self-reinnervation. In combination with the earlier observation of a reversal in the normal relationship of axonal conduction velocity to motor unit tension production in soleus muscle cross-reinnervated by the FDL nerve (slower units larger than fast units; the reverse of normal FDL; Bagust et al. 1981), this suggested to these workers that slow axons might have a competitive advantage over fast axons in reinnervating adult slow muscle fibers.

We here use an intracellular approach to the model of self-reinnervation of the MG muscle in the cat, to explore the limits of regulatory interactions between alpha-motoneurons and the muscle fibers they innervate. Specifically, three questions are addressed. First, are motoneuron and muscle properties reestablished (overall and within motor unit types) following nine months self-reinnervation? Second, is there evidence for a competitive advantage of any motor unit type in reestablishing motor unit size or numbers? Finally, to what extent are muscle unit properties dictated by the innervating motoneurons and vice versa; that is, are the normal relationships between motoneuron and muscle properties restored?

Results

Effects of Cage Time

Since some of the animals in this study were caged for nine-months prior to the acute experiments, it was important to control for any effects of cage-time *per se*. Data were compared from non-caged animals (11 cats, all female, 2.0 to 6.4 kg, mean weight 3.7 kg) and cats caged from 151 to 284 days (5 cats, four females and one male, 2.1 to 3.6 kg, mean weight 2.9 kg). There were no differences in the distribution of motor unit types between non-caged and long-caged cats.

The only differences in motoneuron properties ($p<0.01$, Mann-Whitney U-test) between non-caged and long-caged cats were rheobase and rheobase/input resistance of the population as a whole (across motor unit types), and input resistance in type S motor units (Table 3-2). Similarly, contractile parameters did not differ between non-caged and long-caged cats at the 0.01 alpha level (Table 3-3). We conclude that the effects of cage time were minimal and the non-caged and long-cage groups will be considered together as the normal control group for the remainder of this paper.

Normal Data

Table 3-1 lists values for muscle wet weights and whole muscle twitches for normal and self-reinnervated MG muscles. Normal MG values for muscle weight, muscle weight/body weight, and speed and tension-related parameters were similar to those obtained for cat MG in other studies (Gardiner *et al.* 1978; Mayer *et al.* 1984; Sacks and Roy, 1982, Spector *et al.* 1980).

Table 3-1. Whole Muscle Properties: NORMAL and Nine Month Self-reinnervated. [a,c]

	NORMAL	LONG-RE
Twitch Time-To-Peak [b] (ms)	32±1	33±3
Twitch Half-Rise Time [b] (ms)	11±0	10±0
Twitch Half-Relaxation Time [b] (ms)	22±2	25±2
Twitch Tension [b] (g-wt.)	2123±151	1609±276
Muscle Weight (g)	9.0±1	7.0±1*
Muscle Wt./Cat Wt. (g/kg)	3.0±0	2.3±0*
Twitch Tension/Cat Weight (g-wt./kg)	742±65	488±66*

a. Means±SE (for NORMAL n=14; for LONG-RE n=4).
b. Twitch at muscle length at which maximum tension was obtained.
c. * = significant difference from control ($p<0.05$);

The normal population (non-caged plus caged, see Methods) consisted of 48% type FF units, 4% type FI, 24% type FR, and 24% type S units (Table 3-6). This is similar to that reported by Burke et al. (1973), and Fleshman et al. (1981).

Normal MG muscle contained 56% type FG, 23% type FOG, and 21% type SO fibers (Table 3-6; see also Ariano et al. 1973; Burke and Tsairis, 1973). There were no differences between non-caged and long-caged cats with respect to muscle fiber type distribution.

In cat MG, type FF muscle units are comprised of type FG muscle fibers (or IIb), type FR units of type FOG fibers (IIa), and type S units of type SO fibers (I; Burke and Tsairis, 1973). Table 3-6 estimates relative innervation ratios for each motor unit type.

In general, normal values for motoneuron electrical properties (Table 3-3) and muscle unit contractile properties (Table 3-2) were similar to those reported previously (Burke et al. 1973; Fleshman et al. 1981; Zengel et al. 1985).

Control vs. Reinnervated

Acute physiological experiments were performed on four animals (three female, one male; 2.9 to 3.6 kg, mean 3.4 kg) nine months after the initial surgery. An additional animal (female, 2.7 kg) was used for muscle histochemistry only. The normal distribution of motor unit types was reestablished, confirming results obtained by Burke and colleagues (Burke, 1980; Burke et al. 1979; Dum et al. 1979, in press b) in self-reinnervated cat FDL, and by Gordon and Stein (1982a) in self-reinnervated cat MG (Table 3-6).

Whole Muscle

Following nine months recovery from the initial surgery, MG muscle weights were about 70% of unoperated normal MG. Whole muscle twitch time-to-peak as well as half-rise and half-relaxation times were normal (Table 3-1; Bagust and Lewis, 1974; Buller et al. 1960; Burke, 1980; Burke et al. 1979; Chan et al. 1982; Dum et al. 1979, 1985b; Gordon and Stein, 1982b). In the operated animals muscle weight was significantly reduced as was muscle weight/body weight. Twitch tension was also lower in reinnervated animals ($p<0.05$), but tension /muscle weight was unchanged (Table 3-1). These results are similar to those of Chan et al. (1982), Bagust et al. (1981), and Lewis et al. (1982), but Gordon and Stein (1982b) and Burke (1980) saw complete recovery of muscle weight and twitch tension.

Muscle Unit Contractile Properties.

There were several differences in contractile properties between normal and self-reinnervated animals (Table 3-2). Overall, across motor unit types, mean maximum tetanus was lower in the reinnervated group (23 vs. 35g-wt.; Table 3-2). This trend was still present when maximum tetanus was normalized by body weight, although no longer statistically significant ($p>0.05$). This overall effect reflects the significantly lower maximum tetanic contractions of type FF units (also significant for normalized values). Types FR and S unit tensions were normal. Consistent with the mean values, there were few units with large maximum tetanic contractions in the reinnervated muscles (Fig. 3-1C). We saw no

Table 3-2. Muscle Unit Contractile Properties. (Long-caged vs. Non-caged; Normal vs. Nine month self-reinnervated). [a,b,c]

	FF	FI	FR	S	ALL
TWITCH TIME-TO-PEAK (ms) [d]					
NON-CAGE	29±1 (17)	29±2 (4)	24±1 (10)	50±5 (4)	30±1 (35)
LONG-CAGE	29±1 (39)	26±3 (3)	27±1 (20)	60±4 (10)	36±2 (80)
NORMAL MG	29±1 (56)	28±2 (7)	26±1 (30)	58±4 (22)	34±2 (115)
LONG-RE	28±1 (32)	25±5 (3)	27±1 (17)	55±3 (18)	34±2 (70)
TWITCH TENSION (g-wt.) [d]					
NON-CAGE	19±2 (17)	4±2 (4)	1±0 (10)	0.2±0 (4)	9±0 (35)
LONG-CAGE	17±1 (39)	4±2 (3)	1±0 (10)	0.3±0 (18)	8±1 (78)
NORMAL MG	18±1 (56)	4±1 (7)	1±0 (28)	0.2±0 (22)	9±1 (113)
LONG-RE	10±1 (32)*	2±6 (3)	1±0 (17)	1.0±0 (18)	5±1 (70)*
TWITCH HALF-RELAXATION TIME (ms) [d]					
NON-CAGE	23±2 (17)	30±3 (4)	23±2 (9)	57±11 (3)	26±2 (33)
LONG-CAGE	25±1 (39)	23±1 (3)	27±1 (20)	74±9 (16)	36±3 (78)*
NORMAL MG	24±1 (56)	27±2 (7)	26±1 (29)	74±8 (19)	33±2 (111)
LONG-RE	28±2 (32)	24±5 (3)	25±2 (16)	51±4 (15)	32±2 (66)
MAXIMUM TETANIC TENSION (g-wt.)					
NON-CAGE	66±4 (45)	22±1 (4)	16±1 (25)	9±2 (22)	38±3 (96)
LONG-CAGE	55±3 (39)	18±8 (3)	13±4 (20)	3±1 (17)	31±3 (78)
NORMAL MG	61±3 (84)	20±3 (7)	13±1 (45)	7±1 (39)	35±2 (175)
LONG-RE	37±4 (31)*	20±0 (3)	13±3 (17)	6±1 (16)	23±2 (67)*
FATIGUE INDEX					
NON-CAGE	.04±0 (44)	.59±0 (4)	1.1±0 (25)	1.0±0 (21)	0.6±0 (94)
LONG-CAGE	.03±0 (38)	.34±0 (3)	1.0±0 (20)	1.1±0 (17)	0.5±0 (79)
NORMAL MG	0.0±0 (82)	0.5±0 (7)	1.0±0 (45)	1.1±0 (40)	0.6±0 (174)
LONG-RE	0.1±0 (30)*	0.5±0 (3)	1.2±0 (17)	1.1±0 (13)	0.6±0 (63)

a. * = Significant difference from NON-CAGE at 0.05 level; ** 0.1 level
b. + = Significant difference from NORMAL at 0.05 level: ++ 0.01 level
c. Means ± SE (number of units).
d. Potentiated Twitch.
e. NORMAL = NON-CAGE + LONG-CAGE.

evidence of greatly enlarged motor units with reinnervation (Chan et al. 1982; Gordon and Stein, 1982a,b; but see Rosenfalck and Buchthal, 1970; Yahr et al. 1950). Most studies of reinnervated muscle have reported decreased mean tension with increased variance [Bagust and Lewis, 1974; Burke, 1980 (cat flexor digitorum longus: FDL); Chan et al. 1982 (cat flexor hallucis longus: FHL)]. Distributions of twitch and tetanic tension in this study were similar to those of normals in range for all types except type FF where distributions were shifted to lower values (Fig. 3-1A,C).

Mean potentiated twitch amplitude was smaller in self-reinnervated MG, but not significantly so (Table 3-2). The overall distribution was similar to that of normals, except there were few large twitch units within type FF (Fig. 3-1A). Gordon and Stein (1982b) reported recovery of mean twitch tension, with reinnervated units showing higher variance than normals. Bagust and Lewis (1974), Bagust et al. (1981), and Lewis et al. (1982) found little change in mean twitch tension, although the distribution included a few very large units and many very small units. Part of the difference between their results and ours may be due to their expression of motor unit tension relative to whole muscle tension. Absolute motor unit sizes were not abnormally large compared to normal values.

In general, type FF units produced less tension and type S units more tension than normals (Table 3-2; also true if normalized by cat weight). The increased type S unit twitch amplitude, without increased tetanus resulted in significantly increased twitch/tetanus ratio. We saw no difference in twitch/tetanus ratio between normal and reinnervated MG.

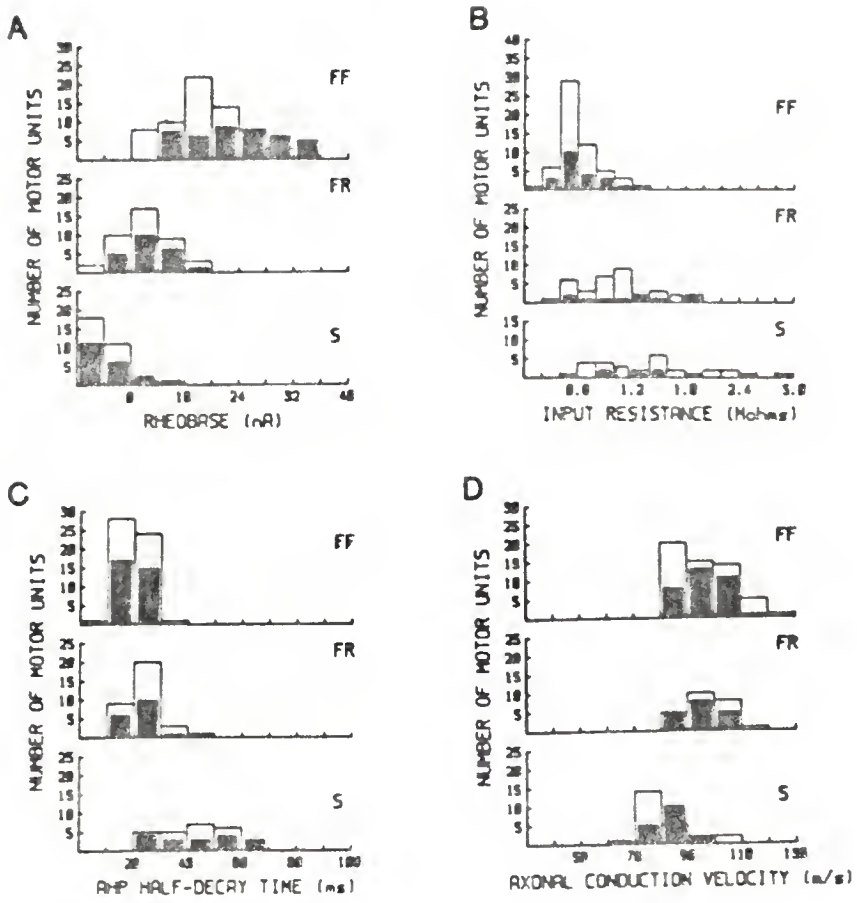

Figure 3-1. Frequency histograms for normal (unfilled) and nine month self-reinnervated (filled) MG motoneuron properties. (A) Rheobase (B) Input resistance (C) AHP half-decay time (D) axonal conduction velocity. See text for explanation.

There were no differences between reinnervated and normal muscle units in twitch time-to-peak (Bagust and Lewis, 1974b; Burke, 1980; Chan et al. 1982; Gordon and Stein, 1982a) or half-relaxation time (Bagust and Lewis, 1974b). There were no differences in the frequency distributions for the reinnervated units' twitch time-to-peak or fatigue index (Fig. 3-1B,D) compared to normals. There was a significant increase in mean fatigue index in type FF units of reinnervated animals (0.06 vs. 0.03), confirming a general impression of a "residual" tension following the fatigue test in these animals (unpublished observations).

Reinnervated motor units were similar to normal units in the pattern of differences between motor unit types for contractile properties (Table 3-4). The exceptions were that reinnervated types FR and S unit fatigue indices did not differ, and the only difference for twitch/tetanus was between types S and FR units.

In reinnervated units the overall correlation between twitch time-to-peak and tetanic tension was -0.47 ($p < 0.0001$), similar to normals (-.40, $p < 0.0001$), and confirming Bagust and Lewis (1974). The correlation was -0.53 within reinnervated type S units ($p < 0.02$; not significant in normals). Types FF and FR units show no correlation between these properties in normals or reinnervated animals.

The overall correlation between twitch time-to-peak and twitch amplitude was weak (-0.32, $p<0.004$) for self-reinnervated units (vs. -.29, $p < 0.003$ in normals) and nonsignificant within any motor unit type in either population. Gordon and Stein (1982a) reported a restoration of the normal negative correlation between twitch time-to-peak and twitch amplitude with reinnervation.

Speed- and fatigue-related properties were normal in mean values and distribution nine months' following section and re-suture of the MG nerve. The relationships between time-to-peak and tension were also normal. The exception to complete restoration of normal properties was that type FF units did not fully recover ability to generate tension.

Motoneuron Electrical Properties

There were no significant differences in motoneuron properties between operated animals and normals ($p<0.01$). At the 0.05 alpha level only rheobase in FF units, and rheobase/input resistance in S units reached significance (Table 3-3). Significant differences between motor unit types in reinnervated motor units were similar to normals (Table 3-4). Exceptions include input resistance, where FR units were not significantly different from FF or S units, and rheobase, where FR and S units differ at $p<0.05$ only.

There was no significant difference in axonal conduction velocity between regenerated and normal MG motoneurons (Table 3-3). This was also seen by Gordon and Stein (1982a) for MG self-reinnervated for 9 months. Kuno et al. (1974b) reported that after 4 months, regenerated MG motoneurons' axonal conduction velocity had not quite recovered to non-caged levels. This difference of results may be due to the shorter recovery time in the Kuno et al. (1974b) study. Consistent with this, Lewis et al. (1978) reported lower than non-caged conduction velocity in self-reinnervated FDL axons at 6 months but not at two years following the initial surgery.

Table 3-3. Motoneuron Electrical Properties. (Long-caged vs. Non-caged; Normal vs. Nine month self-reinnervated).[a,b,c]

	FF	FI	FR	S	ALL
RHEOBASE (nA)					
NON-CAGE	22±1 (40)	18±2 (3)	11±1 (23)	5±0 (20)	16±1 (86)
LONG-CAGE	18±1 (20)	14±1 (3)	10±1 (14)	5±1 (14)	13±1 (61)**
NORMAL MG[d]	20±1 (70)	16±1 (6)	11±1 (37)	5±0 (34)	14±1 (147)+
LONG-RE	24±1 (33)	18±3 (3)	11±1 (18)	6±1 (19)	15±1 (73)+
INPUT RESISTANCE (Mohms)					
NON-CAGE	0.6±0 (33)	0.6±0 (2)	1.0±0 (17)	1.2±0 (15)	0.9±0 (67)
LONG-CAGE	0.7±0 (23)	1.4 (1)	1.1±0 (13)	1.9±0 (13)**	1.2±0 (50)
NORMAL MG[d]	0.6±0 (56)	0.9±0 (3)	1.1±1 (30)	1.5±0 (28)	1.0±0 (117)+
LONG-RE	0.7±0 (22)	0.7±0 (2)	1.2±0 (10)	1.3±0 (11)	1.0±0 (45)+
RHEOBASE/INPUT RESISTANCE (nA/Mohms)					
NON-CAGE	38±2 (32)	28±1 (2)	15±2 (17)	5±1 (15)	23±2 (66)
LONG-CAGE	29±2 (23)	14 (1)	10±1 (13)	3±1 (13)	16±2 (50)
NORMAL MG[d]	35±2 (55)	23±5 (3)	12±1 (30)	4±1 (28)	21±2 (117)+
LONG-RE	40±4 (22)	33±12 (2)	10±2 (10)	6±1 (11)	23±3 (45)+
AHP HALF-DECAY TIME (ms)					
NON-CAGE	21±2 (25)	15±0 (2)	24±1 (17)	43±1 (14)	26±1 (48)
LONG-CAGE	23±1 (29)	22±1 (3)	24±1 (14)	51±4 (14)	30±2 (60)
NORMAL MG[d]	22±1 (54)	19±2 (5)	25±1 (31)	49±3 (28)	28±1 (118)
LONG-RE	20±1 (33)	23±1 (3)	24±2 (18)	44±3 (19)	27±1 (73)
AXONAL CONDUCTION VELOCITY (ms)					
NON-CAGE	99±2 (16)	104±2 (4)	103±2 (7)	85±4 (4)	98±1 (31)
LONG-CAGE	96±1 (39)	95±8 (3)	99±2 (18)	80±2 (20)	92±1 (80)
NORMAL MG[d]	97±1 (55)	100±4 (7)	99±2 (25)	81±2 (24)	94±1 (111)+
LONG-RE	96±2 (33)	94±5 (3)	96±2 (18)	84±2 (19)	90±1 (73)+

a. * = Significance at 0.05 level from NON-CAGE; ** 0.01 level.
b. + = Significance at 0.05 level from NORMAL; ++ 0.01 level.
c. Means ± SE (number of units).
d. NORMAL MG = NON-CAGE + LONG-CAGE.

Table 3-4. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types: NORMAL VS. Nine Month Self-reinnervated MG (LONG-RE).

	NORMAL		LONG-RE	
		P		P
Axonal Conduction Velocity	(F,R)>S	0.01	(F,R)>S>N	0.01
Rheobase	F>R>S	0.01	F>(R,S,N)	0.01
			F>R>(S,N)	0.05
Input Resistance	F<R<S	0.01	F<(S,N)	0.01
			F<R<S<N	0.05
Rheobase/Input Resistance	F>(R,S)	0.01	F>(R,S,N)	0.01
	F>R>S	0.05	F>(R,S,N)	0.05
AHP Half-Decay Time	(F,R)<S	0.01	(F,R,N)<S	0.01
Twitch Amplitude [a,b]	F>(R,S)	0.01	F>(R,S)	0.01
Twitch Time-To-Peak [b]	(F,R)<S	0.01	(F,R)<S	0.01
Twitch HRT [b,c]	(F,R)<S	0.01	(F,R)<S	0.01
Maximum Tetanic Tension [a]	F>(R,S)	0.01	F>(R,S)	0.01
Fatigue Index	F<S<R	0.01	F<(R,S)	0.01
Twitch/Tetanus [d]	F>(R,S)	0.01	(F,S)>R	0.01

a) The same result was obtained with raw data and data normalized for body weight.
b) Potentiated twitch.
c) HRT = half-relaxation time.
d) Twitch/tetanus = unpotentiated twitch/maximum tetanus.

All motor unit types had ranges of AHP half-decay times similar to normals (Fig. 3-2). Kuno et al. (1974b) found that regenerated MG motoneurons had AHP durations that were not significantly longer than normal (82 vs 75ms). In contrast, regenerated soleus motoneurons (virtually all slow in normals) had AHP durations that were significantly shorter than normal (123 vs 150ms). Thus the type S motoneurons of the mixed muscle MG appear to behave differently in this respect than soleus type S motor units following regeneration.

Kuno et al. (1974b) found that 45-60% of five-month regenerated MG motoneurons had values for AHP duration/axonal conduction velocity within the normal range. We found that 96% (44/46) of regenerated MG motoneurons were in the normal range with respect to AHP half-decay time and axonal conduction velocity (Fig. 3-3) and there was little overlap between the distribution of fast and slow units (35/36= 97% of regenerated fast motoneurons in the normal fast range; 13/18 = 72% of regenerated slow motoneurons in the normal slow range). Axonal conduction velocity decreased as AHP half-decay time increased in regenerated motoneurons ($r= -0.42$, $p<0.0001$; vs. -0.52, $p<0.0001$ in normals). Within reinnervated type S units the correlation was -0.69 ($p<0.0001$; -0.51, $p<0.03$ in normals), and there was no relationship for types FF and FR units as in normals.

The distribution of values for rheobase was indistinguishable from that of normals (Fig. 3-2A). The same was true for input resistance, with the possible exception of a lack of large input resistance (>1.5 Mohm) cells in S units of the operated animals (Fig. 3-2B). This may represent an artifact of sampling.

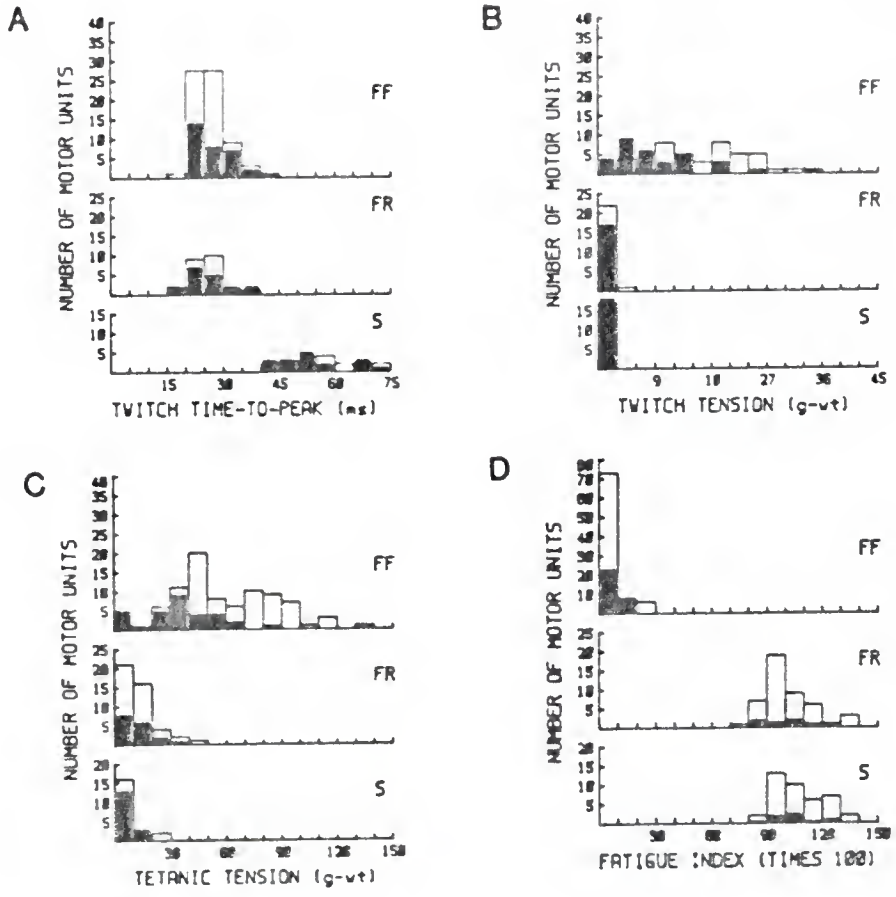

Figure 3-2. Frequency histograms for normal (unfilled) and nine month self-reinnervated (filled) MG muscle unit contractile properties. (A) potentiated twitch amplitude (B) potentiated twitch time-to-peak (C) tetanic tension (D) fatigue index. See text for explanation.

Figure 3-4A shows the relationship between rheobase and input resistance in regenerated motoneurons (3-4B) as compared to normals (3-4A). Figure 3-4B illustrates that, as in normals, motoneurons segregated according to type on the basis of rheobase: input resistance ratio. The correlation between log rheobase and input resistance was -0.63 (normal, -0.60, $p<0.0001$; see also Fleshman et al. 1981; Zengel et al. 1985). No significant correlations were found within any motor unit type in normal or reinnervated units.

The criteria used by Zengel et al. (1985) were applied to these data to estimate motoneuron type (AHP half-decay time < 30ms = fast, AHP half-decay time > 30ms = slow; rheobase/input resistance <7 = S, rheobase/input reistance > 18 = FF, with FR motoneurons having rheobase/input resistance between 7 and 18). Based on these criteria there was an 86% agreement in normal MG, between motoneuron type and motor unit type (determined by contractile properties). When these same criteria were applied to the reinnervated motor units, there was an 84% agreement between motor unit type (contractile) and motoneuron type (above criteria).

Thus, after nine months, regenerated MG motoneurons had normal mean values and frequency distributions for membrane electrical properties. Relationships between motoneuron electrical properties were normal, and motoneuron type (defined by electrical properties) accurately predicted motor unit type (defined by contractile properties).

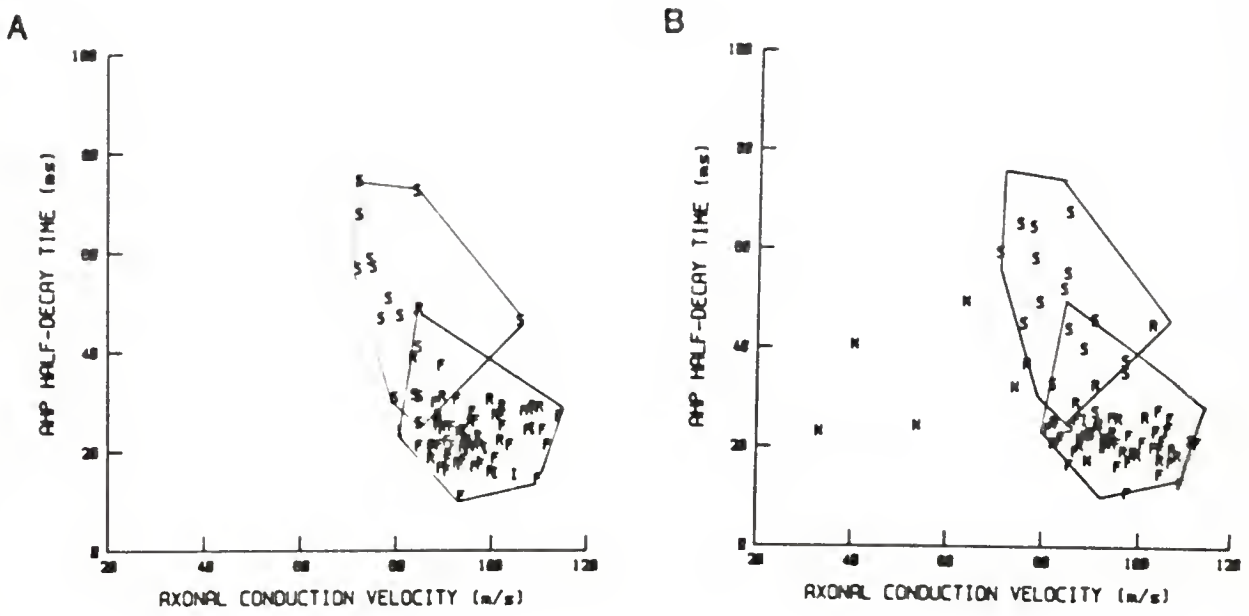

Figure 3-3. Relationships between AHP half-decay time and axonal conduction velocity in normal and nine month self-reinnervated MG motoneurons. (A) Normal MG. Note overlapping but largely separate distributions for slow (S) and fast (F=FF, R=FR, I=FI) motoneurons. Solid lines outline the distribution of slow (upper) and fast motoneurons. (B) Nine months self-reinnervated MG. Note precise reestablishment of distributions for slow (S) and fast (F=FF, R=FR, I=FI) motoneurons. Most non-contracts (N) fall outside the normal range. Solid lines outline the distribution of slow (upper) and fast (lower) motoneurons in controls.

Non-Contracts

Unlike those in the normal population, there were a few motoneurons (8/81) in the self-reinnervated animals which, when stimulated, did not elicit measurable muscle contractions. We have termed these cells "non-contracts" as we cannot rule out their being a-fusimotor in nature (Gregory et al. 1982). It is unlikely that these are gamma motoneurons as they received monosynaptic input from the LG-S nerve (Table 3-5). A subpopulation within the regenerated motoneurons which did not elicit muscle contraction has been observed previously (Bagust and Lewis, 1974; Gordon and Stein, 1982b; Kuno et al. 1974b; Dum et al. in press).

Mean values and raw data for individual non-contracts' electrical properties are seen in Table 3-5. In axotomized motoneurons, mean axonal conduction velocity and the mean and range for rheobase decreased, while mean input resistance was increased (Chapter 4; Gustafsson, 1974; Gustafsson and Pinter, 1984; Kuno et al. 1974a). Mean AHP half-decay time is little changed with axotomy, but the range was compressed from both the high and low ends (Chapter IV; Gustafsson, 1974; Gustafsson and Pinter, 1984; Kuno et al. 1974a). Some of the non-contracts at nine months' self-reinnervation fit this pattern (cells 6,7,and 8) while others show various combinations of properties within and outside the range for normal motoneurons. Six of the eight non-contracts fell outside the normal range of values for the ratio AHP half-decay time: axonal

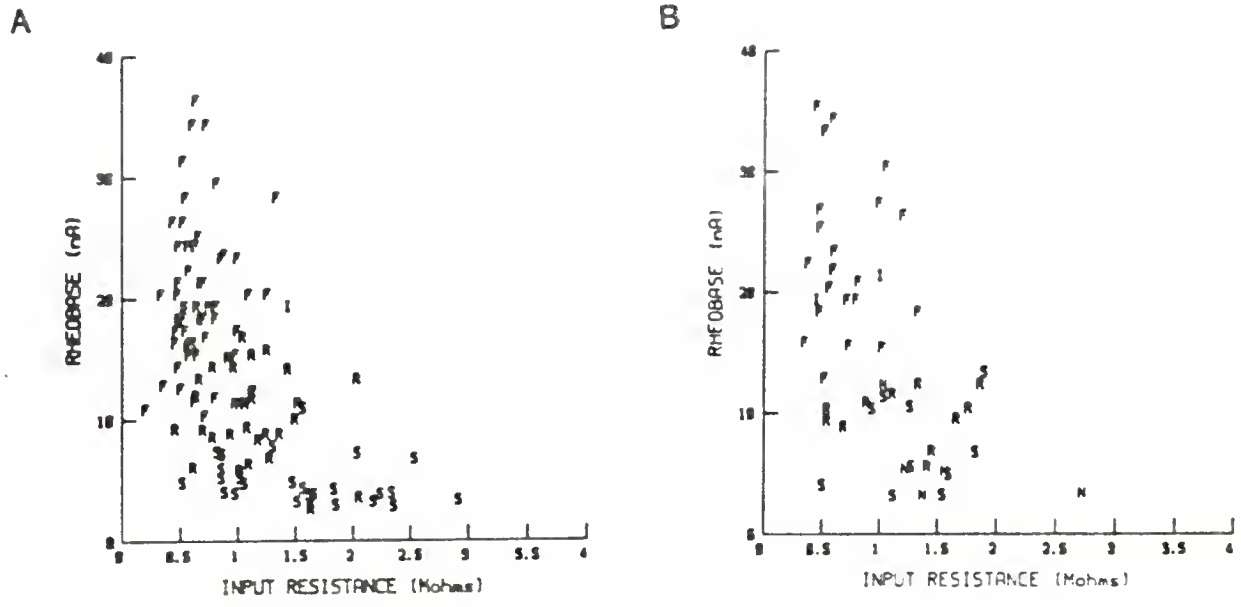

Figure 3-4. Relationships between rheobase and input resistance in normal and nine month self-reinnervated MG motoneurons. F= type FF units, I=type FI, R=type FR, S=type S units, N=non-contract. (A) Normal MG. Note segregation by motor unit type. (B) Nine months self-reinnervated MG. Segregation by motor unit type is re-established. The lack of high input resistance type S units may reflect sampling variability.

conduction velocity (Fig. 3-4). It is possible that some of the cells with relatively normal properties were injured in dissection or have made contact with intrafusal muscle fibers (Gregory et al. 1982).

Relationships Between Motoneuron and Muscle Unit Properties

In normal animals, the strongest correlation between a motoneuron electrical property and muscle unit contractile property was between AHP half-decay time and twitch time-to-peak (r= 0.74, p<0.0001 overall; 73). This relationship was also present in reinnervated units (0.42, p<0.0001). We saw no significant relationships within motor unit types in operated or normal animals, in contrast to Zengel et al. (1985), who found a significant correlation within S units of MG. Huiszar et al. (1977) also reported a significant correlation between AHP duration and twitch time-to-peak for soleus motor units.

In operated animals, as in normals, the correlations between axonal conduction velocity and twitch (r = 0.23, p<0.04) or tetanic tension (0.37, p<0.001), normalized by cat weight and expressed as log tension, were weak (0.41, p<0.0001 for twitch; 0.44, p<0.0001 for tetanus; 2,29). There were no significant correlations between these variables within any motor unit type in reinnervated units. In normals the relationships were significant within type S units only (0.58 for twitch, ns for tetanus).

The correlation between axonal conduction velocity and twitch time-to-peak was -0.50 overall (p<0.0001) and -0.53 for type S units (p<0.02). This compares to -0.52 and ns in normals. Gordon and Stein (1982a) also showed restoration of the normal negative correlation

Table 3-5. Data for Non-Contracts (Nine Month Self-Reinnervation Model.)

CELL	RHEO[a]	RN[b]	RHEO/RN[c]	HALFTIME[d]	C.V.[e]	PSP[f]
1	6	X	X	36	76	1.4
2	3	X	X	17	89	X
3	8	X	X	31	73	1.0
4	5	1.2	4.2	21	87	1.6
5	12	1.0	12.0	23	53	1.0
6	3	1.3	2.3	40	40	1.5
7	5	1.5	3.3	22	32	2.3
8	3	2.7	1.1	49	63	0.4

MEAN VALUES ±SE (NUMBER OF UNITS)

	NON-CONTRACTS	REGENERATED	CONTROL
RHEOBASE (nA)	7±1 (8)	15±1 (73)	14±1 (147)
RN [b] (Mohm)	2±0 (5)	1±0 (45)	1±0 (117)
RHEO/RN [c] (nA/Mohm)	5±4 (5)	23±3 (45)	21±2 (116)
HALFTIME [d] (ms)	30±4 (8)	27±1 (73)	28±1 (111)
AXONAL C.V. [e] (m/s)	64±7 (8)	90±1 (73)	94±1 (118)

a) RHEO = rheobase
b) RN = input resistance
c) RHEO/RN = rheobase/input resistance
d) HALFTIME = AHP half-decay time
e) C.V. = conduction velocity
f) PSP = composite monosynaptic Ia EPSP from LG-S nerve

between axonal conduction velocity and twitch contraction time in self-reinnervated cat MG. In contrast, Bagust and Lewis (1974) reported that the normal inverse relationship between time-to-peak and axonal conduction velocity was lost in reinnervated muscles. While it is not clear what can account for these differences, the present surgeries were all performed on adult cats whereas Bagust and Lewis (1974) operated on young cats of around one kg in weight.

In summary, the relationships between electrical properties of self-regenerated motoneurons and contractile properties of self-reinnervated muscle units were similar to those which exist between normal motoneurons and muscle units.

Muscle Fiber Histochemistry and Fiber Areas

Mean cross-sectional areas of nine months' self-reinnervated MG muscle fibers were 3098um^2 for type FG fibers (3873um^2 normal), 2355um^2 for type FOG (2264um^2 normal), and 2296um^2 for type SO fibers (1972um^2 normal), with an overall average of 2738um^2 (88% of normal = 3104um^2; Table 3-6). The areas of types FOG and SO fibers were similar to normal values but type FG fiber areas were significantly below normal levels. The reduced overall mean area was due to this lack of type FG recovery.

Burke (1980) found that after one year, the normal distribution of muscle fiber types was present in FDL, although "type-grouping" (Dubowitz, 1967; Romanul and Van Der Meulen, 1967) was evident (Burke, 1980). We found 54% type FG fibers, 15% type FOG fibers, and 31% type SO fibers (56;23;21 in normals) and fiber "type-grouping" was evident (Fig. 3-5). This overall distribution was significantly different from normal (chi-square test, $p<0.05$). There was an increase in the

Table 3-6. Motor Unit Types, Muscle Fiber Types, and Innervation Ratios (Normal and Nine Month Self-Reinnervated).

MUSCLE FIBER TYPES

Histochemical Composition

	FG	FOG	SO	n/N [a]
NORMAL	56	23	21	4200/7
LONG-RE	54	15	31	3000/5

Observed Mean Fiber Area (um^2)

				ALL
NORMAL	3873	2264	1972	3104
LONG-RE	3098	2355	2296	2738

Est. Number of Muscle Fibers. [b]

NORMAL	68560	28160	25710	122400
LONG-RE	58320	16200	33480	108000

EQUIVALENT MOTOR UNIT TYPE

% Of Population

	FF+FI	FR	S	n/N [a]
NORMAL	48+4	24	24	176/16
LONG-RE	46+4	24	26	72/4

Est. Number in Pool

				ALL
NORMAL	134+11	67	67	280
LONG-RE [c]	122+11	64	69	265

CALCULATED VALUES

Relative IR [d]	FF+FI	FR	S
NORMAL	1.1	1.0	0.9
LONG-RE	1.1	0.6	1.2

a. Number of Cells/Number of Animals.
b. (Physiological Cross-Sectional Area)/Mean Fiber Area.
c. Estimated as 280-[(280) (% Non-Contracts)].
d. Relative Innervation Ratio = % Motor Unit Type/% Muscle Fiber Type.

proportion of type SO muscle fibers, and a decrease in fast muscle fibers as a group (types FOG+FG). This alteration of proportions of muscle fiber types was not as extensive as that reported by Lewis et al. (1982), however, who presented histochemical data for one self-reinnervated FHL, after over two years' recovery. In that muscle 80% of the fibers were type I (equivalent to type SO) compared to the normal FHL which is 86% type II (Ariano et al. 1973).

Physiological Cross-Section and Innervation Ratios

The physiological cross-section (CSA) of the whole MG muscle was estimated as follows (ref. Dum et al. 1982):

$$CSA = \frac{(\text{muscle weight})(\cos A)}{(\text{muscle density})(\text{muscle fiber length})}$$

Muscle density was assumed to be 1.06g cm^{-3} (Mendez and Keys, 1960). Values for A (angle of pennation) and muscle fiber length were assumed to be 21^{o} and 21mm, respectively, for both normal and reinnervated muscles (Sacks and Roy, 1982). For the reinnervated muscle we estimate physiological cross-section as $3.0cm^2$ ($3.8cm^2$ in normals).

The number of muscle fibers in MG was estimated as physiological cross-section/mean muscle fiber area (Burke and Tsairis, 1973). Mean muscle fiber area was estimated as the sum of the area for each muscle fiber type, multiplied by the frequency of that type in the population. For normals, we estimated the number of muscle fibers in MG as 122,400. This is lower than the 170,000 estimated by Burke and Tsairis (1973) for cat MG, presumably they obtained higher values for a and fiber length, as their value for mean fiber area was higher ($3426um^2$ calculated from Burke and Tsairis, 1973, vs. $3104um^2$ in this study).

The overall average innervation ratio (mean number of muscle fibers innervated by an individual motoneuron; Eccles and Sherrington, 1930) is calculated as the ratio of the number of muscle fibers in MG (estimated as 122,400) to the number of MG motoneurons (280: Boyd and Davey, 1968; Burke et al. 1977). In this study average innervation ratio for normal MG was 436 (vs. 607: Burke and Tsairis, 1973). For reinnervated MG we estimate average innervation ratio to be 429 (108,000/252; estimated numbers of both muscle fibers and innervating axons were decreased; Table 3-6). The relative innervation ratio for each type is the frequency of a given muscle fiber type divided by the frequency of the corresponding motor unit type (Dum et al. 1982; their eq.2).

Relative innervation ratios for reinnervated types FF+FI motor units were 1.1 (vs. 1.1 normals), 0.6 for type FR units (vs. 1.0, although Burke and Tsairis, (1973) reported 0.7 in normal MG), and 1.2 in type S units [vs. 0.9 in normals; 0.9 in Burke and Tsairis, 1973). This may reflect some competitive advantage of S units in capturing and/or maintaining contacts with muscle fibers, although not to the extent reported by Lewis et al. (1982).

Mean motor unit tetanic tensions (Table 3-2) recovered to normal levels in type FR units despite reduced innervation ratios (see below) suggesting increased specific tension (Table 3-6). Bagust et al. (1981) reported altered muscle specific tension following reinnervation and cite Hoh (1974) as showing myofibril packing density was influenced by innervation. Type S units recover to 67% of normal tension levels despite increased numbers of fibers and unchanged area (suggesting lower specific tension, Table 3-6). Type FF units recovered to only 64% of

normal tetanic tension (Table 3-2), primarily due to decreased muscle fiber area (no change in innervation ratio).

Fig. 3-5 shows a single glycogen-depleted type FF motor unit from a nine month self-reinnervated MG. Note the "clumped" nature of the distribution and the homogeneity of muscle fiber type. There were 141 fibers counted in this unit, in the section with the maximal number of depleted fibers. All were FG fibers, confirming previous studies (Burke, 1980; Gauthier et al. 1983; Kugelberg et al. 1970), in that muscle fiber type was homogeneous within a single motor unit following long-term reinnervation. Burke and Tsairis (1973) estimated that counting depleted fibers from a single section of normal MG sampled 50%-75% of the whole unit. From this relation we estimate that the unit in Fig. 3-5 contained 212-284 muscle fibers. This unit had a motoneuron rheobase of 32, muscle twitch time-to-peak of 30ms, 4.4 g-wt. twitch tension, 25 g-wt. tetanic tension, exhibited 'sag', and had a fatigue index of 0.07. A second glycogen depleted type FF unit had 365 fibers in the section with maximal number (estimate of 548-730 muscle fibers). Rheobase was 16 and twitch time-to-peak was 20ms. Tension data are unavailable. Again all fibers were type FG. This unit also exhibited type-grouping, but not to the extent of the unit in Fig. 3-5. The innervation ratios of these depleted FF units fall within the range reported by Burke and Tsairis (1973) for normal MG.

Since calculated innervation ratios were increased for type S motor units (Table 3-6), type S motoneurons may be at advantage in innervating and maintaining connections with muscle fibers following reinnervation of a mixed muscle. Gycogen-depleted type FF units (n=2) fell within the

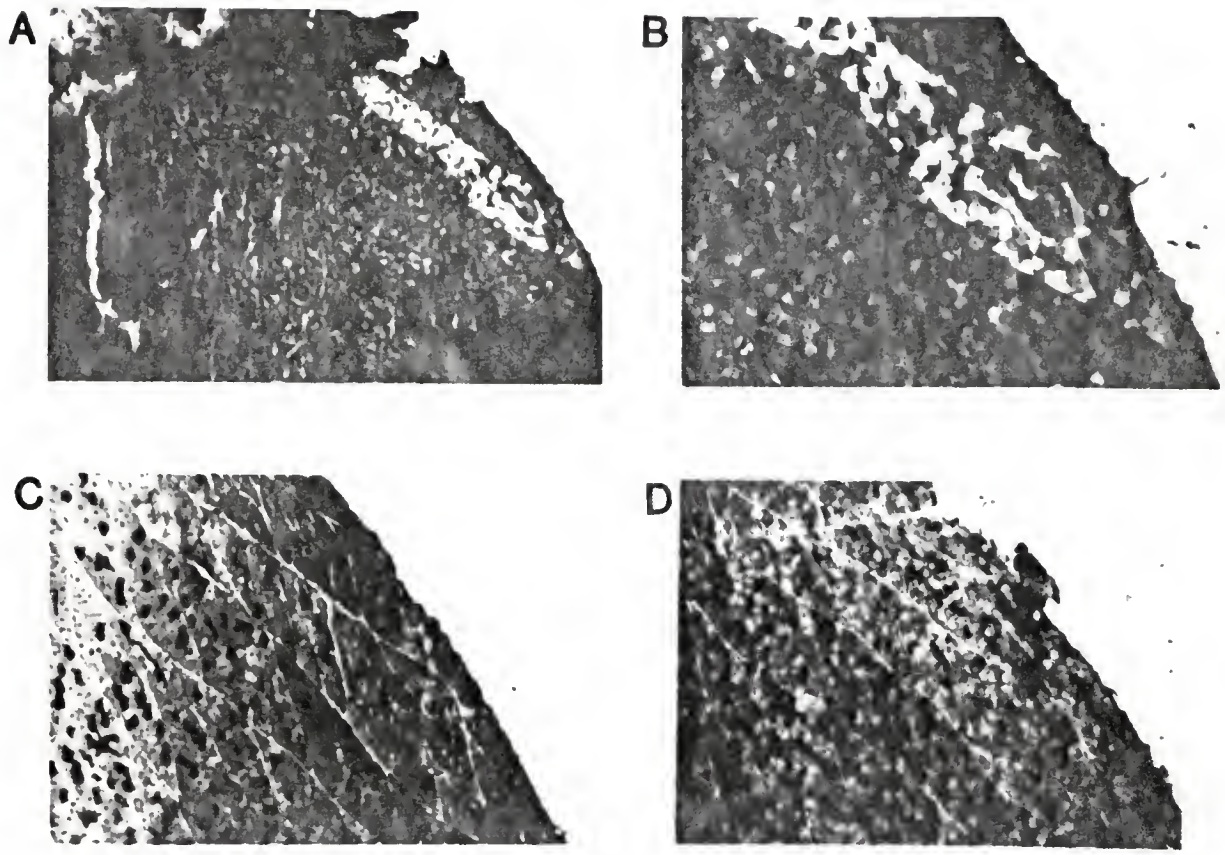

Figure 3-5. Photomicrographs of a single glycogen-depleted type FF motor unit from a nine month self-reinnervated MG. (A) The entire unit (PAS stain for glycogen, 70X) (B) PAS stain (100X) (C) myosin ATPase, pH 4.3 (100X) (D) NADH-D (100X).

normal range for innervation ratios of unoperated normal type FF motor units.

Discussion

In this study we tested whether motoneuron electrical properties, muscle unit contractile properties, and the normal relationships between them, would be restored nine months following section and re-anastomosis of the MG muscle nerve in cats.

We have shown that the normal proportions of motor unit types are re-established in self-reinnervated muscles (Burke, 1980; Gordon and Stein, 1982a). Motoneuron electrical properties (rheobase, input resistance, AHP half-decay time, and axonal conduction velocity), as well as correspondence between motoneuron (electrical) type and motor unit (contractile) type, are normal following nine months reinnervation. As in controls, self-regenerated MG motoneurons segregate by motor unit type with respect to the ratio rheobase:input resistance. This ratio, in conjunction with AHP half-decay time, predicts motor unit types in self-reinnervated as well as control MG (Zengel *et al.* 1985).

We found that axonal conduction velocity recovers to normal levels following reinnervation (Burke, 1980; Burke *et al.* 1979; Gordon and Stein, 1982a; Kuno *et al.* 1974b; Lewis *et al.* 1978). Kuno *et al.* (1974b) showed that after five months self-reinnervation, action potential overshoot, resting membrane potential, and conduction velocity were normal or nearly normal in MG, while AHP duration had not recovered completely. Our study confirms these earlier reports, and extends the documentation of recovered motoneuron properties to motoneuron rheobase, input resistance, and their relationship. Previous works have not

distinguished between motor unit types with respect to motoneuron properties [except axonal conduction velocity (Burke, 1980; Gordon and Stein, 1982a) and AHP duration (Burke, 1980)]. We also found motoneuron properties within each motor unit type in self-reinnervated motor units to be similar to properties in control motor units of the same type.

Besides the above-mentioned relationship between rheobase and input resistance, other relationships between motoneuron properties in long-term self-reinnervated motor units are similar to normal MG. Overall negative correlations were seen between rheobase and input resistance, and between axonal conduction velocity and input resistance or AHP half-decay time. As reported by Kuno et al. (1974a,b), fast and slow motoneurons tend to segregate on plots of conduction velocity vs. AHP half-decay time (AHP duration in Kuno et al. 1974a,b), in both control and regenerated motoneurons (Fig. 3-4). Kuno et al. (1974a,b) defined 'fast' as MG, and 'slow' as soleus motoneurons. We extend this observation to fast and slow motor unit types within one mixed muscle, MG, and document the complete recovery of this relationship after nine months reinnervation. The lack of correlation between these variables within motor unit types suggests that overall correlations may be a consequence of the presence of different motor unit types, each with characteristic properties, as in normal motoneurons (Fleshman et al. 1981).

Self-reinnervated muscle units did not differ from normal with respect to speed (isometric twitch time-to-peak, half-relaxation time) or fatigue resistance (although type FF units were slightly less fatigable). The exception to complete recovery of overall mean values

for motor unit properties was an overall decline in mean maximum tetanic tension, especially for type FF units [also seen in reinnervated FDL (Burke, 1980) and in flexor hallucis longus (Chan et al. 1982)]. This failure to recover tension was due to lack of recovery of muscle fiber area (type FG fibers). We found no evidence for greatly enlarged motor units in operated animals (Chan et al. 1982; Dum et al. 1979; Gordon and Stein, 1982a,b). These data suggest that neural influence may be more effective in regulating muscle twitch speed and fatigue resistance than muscle unit tension during self-reinnervation in adult cats.

Twitch speed and fatigue resistance are the parameters which define motor unit type (Burke et al. 1973). These parameters are also the focus of most chronic stimulation experiments (reviewed in Pette, 1984; Salmons and Henrikson, 1981), although Lomo et al., (1980) reported influence of frequency of activation on force generation by chronically denervated and stimulated rat soleus muscles. Spector (1984) compared denervated rat muscles to muscles completely inactivated by TTX, and reported that while changes in speed of shortening could be accounted for by activity alone, muscle weight, force generation, and specific tension appeared to be regulated by release of a trophic substance as well. Our data are consistent with differential regulation of force and speed-related muscle properties by the motoneuron.

Relationships between contractile properties after nine months self-reinnervation were also similar to normal. Motor unit twitch time-to-peak was negatively correlated with twitch amplitude and with maximum tetanic tension across the whole population of reinnervated

units, similar to controls (Bagust and Lewis, 1974). We found no correlations between contractile properties within motor unit types (except twitch time-to-peak vs. tetanic tension in type S units).

The similarity between normal and self-regenerated motor units in motoneuron electrical properties, and in motor unit type distribution, argues against a selective advantage for any motor unit type in terms of contacting muscle fibers and surviving, although the number of fibers innervated by a given unit type may vary (see below). Thus although 10% of the axons made no functional reconnection with extrafusal muscle fibers, there was no systematic loss of motoneurons of any type, and the original proportions of each motor unit type were restored by nine months self-reinnervation.

The close correspondence between motoneuron "type" and motor unit type, described above, suggests that proper match-ups between motoneuron and muscle properties are reestablished at the level of single units, following long-term self-reinnervation of MG. This can also be seen in the overall correlations between axonal conduction velocity and twitch or tetanic tension, axonal conduction velocity and twitch time-to-peak, and between AHP half-decay time and twitch time-to-peak. This latter relationship was the strongest between any motoneuron and muscle property in both normal and operated animals. These correlations are weak or absent within motor unit types, suggesting that the overall correlations can be explained by the presence of different types of motor units in self-reinnervated as well as normal MG.

The AHP half-decay time (or duration) is closely related to the motoneurons' steady-state firing frequency (Kernell, 1965;

Gustafsson, 1974). The correlation between AHP half-decay time and twitch time-to-peak may reflect the control of muscle contractile speed by pattern and quantity of activation (reviewed in Salmons and Henrikson, 1981; Pette, 1984). The question still remains as to whether this match-up is dictated solely by the motoneuron to the muscle fibers (as suggested by fiber type grouping of units and results from experiments using chronic electrical stimulation of muscle), or whether there are also retrograde influences by the muscle on the motoneuron (Czeh et al. 1978).

Muscle fiber types were present in nearly normal proportions after long-term self-reinnervation of MG, although there was an increase in type SO muscle fibers and a corresponding decrease in the proportion of fast (FG+FOG) muscle fibers in reinnervated MG. "Type grouping" of fiber types was evident (Dubowitz, 1967; Romanul and Van Der Meulen, 1967). These data are generally consistent with Chan et al. (1982), Burke (1980) and Gauthier et al. (1983), who reported restoration of normal proportions of muscle fiber types with reinnervation. In contrast, Lewis et al. (1982) presented histochemical data for one cat FHL muscle, reinnervated for three years, which was 80% type I (equivalent to type SO) fibers, as compared to a normal 85% type II (FG and FOG). This result, in combination with their finding of slow units being larger than fast units in soleus cross-reinnervated by the mixed FDL nerve, led these authors to propose that slow axons may have a competitive advantage in reinnervation. Our histochemical data show a slight trend in the direction of increased numbers of SO fibers, but not of the

magnitude reported by Lewis et al. (1982) and perhaps not outside of normal variation between animals (real and due to sampling).

The combined data on innervation ratios, muscle fiber type proportions, and motor unit tension production also suggest that there could be a slight competitive advantage for type S axons in reinnervation, as suggested by Lewis et al. (1982). Perhaps within the type S population, recovery of neuromuscular transmission occurs more rapidly when the proper match-up between type S motoneuron and SO fiber is achieved, while fast axons are at a disadvantage unless they innervate an originally fast muscle fiber. This was suggested by Ip and Vrbova (1983) for reinnervation of soleus muscle in rats and could account for the small alteration in motor unit size in the present study. Ip and Vrbova (1983) showed that either soleus' own nerve or a foreign nerve were eventually able to innervate soleus muscle successfully. At early times following the initial surgery, however, soleus' own nerve induced more tension in the muscle, formed more synapses with the original endplates, and could follow high-frequency electrical activation more securely. They noted that the original post-synaptic specialization remains for some time following denervation, and may retain its original properties. Finally, transmission might initially be most effective when the presynaptic terminal is of the same type as the post-synaptic specialization.

Conclusions

Nine months following self-reinnervation, MG motor units have properties remarkably similar to normal adult MG. In addition, the relationships between motoneuron properties and muscle unit properties

are normal. Slow units may have some advantage in making or retaining contacts with muscle fibers, although there is no strong evidence for this conclusion. Fiber type grouping and the restoration of motor unit properties and relationships suggest a major role for the motoneuron in dictating muscle unit properties. The motoneuron may regulate muscle unit speed- and fatigue-related properties differently than muscle unit force generation. The latter property may indicate a limit to motoneuron regulatory influence. Information concerning the nature of neural influence on muscle force is of interest with respect to the observed recruitment of motor units in order of increasing force (Stuart and Enoka, 1983; Zajac and Faden, 1985) and controversies over the neural substrate for such a recruitment pattern (Henneman, 1980a; Sypert and Munson, 1981; Stuart and Enoka, 1983).

This study cannot directly assess whether or not the muscle also effects motoneuron properties, or whether one type of motoneuron or another is better able to make early contact with muscle, or recover its properties more rapidly. Subsequent chapters will address these questions by utilizing surgical cross-reinnervation of muscles with different muscle fiber type distributions, and by looking at the time course of recovery of motoneuron and muscle unit properties during self- and cross-reinnervation.

CHAPTER IV
AXOTOMY AND THE TIME COURSE OF SELF-REINNERVATION OF MG

Introduction

CHAPTER III described virtually complete recovery of cat MG motoneuron electrical properties, muscle unit contractile properties, and the relationships between them, following nine months self-reinnervation. One exception was that type FF motor units failed to regain control tensions, apparently due to a failure of type FG muscle fiber areas to recover. Based upon proportions of muscle fiber types and calculated innervation ratios, it was suggested that type S motor units might have an advantage over type FR units in the ability to make and/or maintain functional connections with muscle fibers.

Very little is known about motor unit properties during the process of reinnervation of skeletal muscle because most studies have focused upon the end-point, when all changes would presumably be completed. Gordon and Stein (1982a,b) used a chronic paradigm to measure motor unit twitch tension and axonal action potential amplitude (extracellular) for cat MG at various times after nerve section and repair. They found that, at the earliest stages of reinnervation, twitch tensions were low, a sizable number of axons did not elicit muscle tension, and the normal relationships between muscle speed and tension, and axonal action potential amplitude and twitch tension, were lost. When whole muscle tension reached about 50% of the value eventually attained, the

relationships between twitch time-to-peak and tetanic tension returned (3 mos. post-operative). After this time they saw no further increase in the estimated number of motor units and suggested that the remaining increase in whole muscle tension was due to increased muscle fiber areas. The relationship between axonal action potential amplitude and twitch time-to-peak returned also (at about three months, although not at control levels until six months). Gordon and Stein (1982a,b) did not attempt to classify units by motor unit type until the final acute experiment, at about one year post-surgery.

Kuno et al. (1974b), studying cat MG and soleus motoneurons, reported that at early stages of reinnervation many motoneurons did not elicit muscle contraction. They reported that these cells did not differ in electrical properties from motoneurons which did elicit muscle tension. They suggested that functional reconnection was neither a necessary nor a sufficient condition for restoration of motoneuron electrical properties. In contrast, Gordon and Stein (1982b) also reported that a group of axons did not make functional reconnection with the muscle, but that in these axons, axonal action potential amplitude did not recover from axotomized levels.

On the basis of histochemistry of self-reinnervated flexor hallucis longus (FHL) and a reversed relationship between axonal conduction velocity and twitch tension in soleus muscle cross-reinnervated by flexor digitorum longus (FDL) nerve, Lewis et al. (1982) hypothesized that slow motor units might have a selective advantage in reinnervation of muscle (see also Ip and Vrbova, 1983).

The purpose of the present study was to examine the time course of self-reinnervation of cat MG to determine: (1) What happens to motoneuron properties following axotomy? From what level must recovery occur? (2) Is functional reconnection with muscle necessary and/or sufficient for recovery of motoneuron electrical properties? (3) At what stage are motor unit types first recognizable? How does this relate to the degree of recovery of motoneuron properties, muscle contractile properties, and their relationships? (4) Until what stage are new motor units being formed? (5) Is any motor unit type at advantage in reinnervating MG? (6) Are there parallels between the time couse of reinnervation and motor unit ontogeny?

Results

Whole Muscle

Table 4-1 lists values for muscle wet weights and whole muscle twitches for all treatment groups where muscle twitch tension could be elicited by nerve stimulation. After the MG nerve was severed there was muscle atrophy. At the earliest stage of reinnervation examined (low-re; two cats), muscle weight and tension were less than half of control values. At this stage isometric twitch time-to-peak was prolonged, as was half-rise time and half-relaxation time.

The medium self-reinnnervation (med-re; two cats) category was defined in terms of recovery of tension. These MG muscles had maximum twitch tensions of over one half of nine month self-reinnervated

Table 4-1. Whole Muscle Properties: Time Course of Self-Reinnervation. [a]

	NORMAL	LOW-RE	MED-RE	LONG-RE
Twitch Time-To-Peak [b] (ms)	32±1	46±14	34±0	33±3
Twitch Half-Rise Time [b] (ms)	11±0	19±3	13±0	10±0
Twitch Half-Relaxation Time [b] (ms)	22±2	70±2	38±8	25±2
Twitch Tension [b] (g-wt.)	2123±151	77±37	893±11	1609±276
Muscle Weight (g)	9.0±1	5.0±0	4.4±1	7.0±1
Muscle Wt./Cat Wt. (g/kg)	3.0±0	1.6±0	1.5±0	2.3±0
Twitch Tension/Cat Weight (g-wt./kg)	742±65	24±11	302±9	488±66

a. Means±SE (for NORMAL n=14; for LOW-RE n=2; for MED-RE n= 2; for LONG-RE n=4).
b. Twitch at muscle length at which maximum tension was obtained.

(long-re) values (maximum twitch >1000g-wt tension). This group was also characterized by time after the initial surgery (63 and 71 days). By this stage values for contraction time (time-to-peak, half-rise, half-relaxation) had recovered to normal or near normal levels. Muscle weights were about 60% of long-re (significant at aLPHA = 0.001). Data for the nine months reinnervated and normal MG are from CHAPTER III.

One animal (FE17), for which we only have whole muscle contractile and histochemical data, was intermediate between the low-re and med-re groups in terms of recovery of tension. This animal was sacrificed at 52 days post-surgery. Maximum whole muscle twitch was 281 g-wt.and time-to-peak was 6 ms. Muscle weight was 5.5g and estimated physiological cross-section was 2.1 cm^2.

A second animal (FE12) also exhibited recovery to a level intermediate between the low-re and med-re stages after only 34 days. This animal's whole muscle twitch was closer to the med-re level (maximum twitch of 653 g-wt., 30 ms time-to-peak). The mass of MG was 7.4g and physiological cross-section was estimated at 3.1 cm^2. Motor unit data were obtained from this animal.

These two animals indicate that recovery was a continuous process, with considerable variability in the rate of recovery for different animals. Data obtained from the other animals were generally consistent at a given defined stage.

Axotomy

To examine the physiological effects of axotomy upon MG motoneurons, four animals were examined three to five weeks (25, 26, 33 and 35 days) following nerve section. No tension could be elicited from the MG muscle by MG nerve stimulation, nor were electomyographic responses seen. Although it is not known whether there were connections which were too weak to elicit tension, structural regeneration of the nerve terminals is not necessarily immediately accompanied by functional neural transmission (Birks et al. 1960; but see Carmignoto et al. 1983).

The mean values for motoneuron electrical properties are seen in Table 4-2. Frequency histograms for motoneuron electrical properties following axotomy are seen in Figures 4-1 and 4-2. There was a marked decrease in axonal conduction velocity in axotomized motoneurons (Eccles et al. 1958; Kuno et al. 1974a,b; Hoffer et al. 1979; Milner and Stein 1981; Cragg and Thomas, 1961; Mendell et al. 1976; Kiraly and Krnjevic, 1959; Gallego et al. 1979b). The range of values for axonal conduction velocity was similar to control, although shifted to lower values (Fig. 4-2B). This decreased conduction velocity is believed to be due primarily to decreased axonal diameter (Gutman and Sanders, 1943; Sanders and Young, 1946), as well as shorter internodal distance and altered ion channel distribution (Beery et al. 1944; Cragg and Thomas, 1964; Ritchie, 1982; Kocsis et al. 1982; Kocsis and Waxman, 1983). Myelin thickness does not change markedly with axotomy (Gillespie and Stein, 1982).

Table 4-2. Motoneuron Electrical Properties: Axotomy and Time Course of Self-reinnervation.[a,b,c]

	FF	FI	FR	S	ALL
RHEOBASE (nA)					
NORMAL MG	20±1 (70)	16±1 (6)	11±1 (37)	5±0 (34)	14±1 (147)+
NO-RE					4±0 (67)*
LOW-RE					5±1 (35)*
MED-RE	29±3 (3)	16±2 (9)	11±2 (13)	3±1 (8)	10±1 (33)*
LONG-RE	24±1 (33)	18±3 (3)	11±1 (18)	6±1 (19)	15±1 (73)+
INPUT RESISTANCE (Mohms)					
NORMAL MG	0.6±0 (56)	0.9±0 (3)	1.1±1 (30)	1.5±0 (28)	1.0±0 (117)+
NO-RE					2.6±0 (60)*
LOW-RE					2.4±1 (35)*
MED-RE	0.7±0 (3)	0.8±0 (7)	1.7±0 (12)*	1.9±0 (7)	1.8±0 (29)*+
LONG-RE	0.7±0 (22)	0.7±0 (2)	1.2±0 (10)	1.3±0 (11)	1.0±0 (45)+
RHEOBASE/INPUT RESISTANCE (nA/Mohms)					
NORMAL MG	35±2 (55)	23±5 (3)	12±1 (30)	4±1 (28)	21±2 (117)+
NO-RE					2±0 (59)*
LOW-RE					3±0 (35)*
MED-RE	52±18 (3)	22±4 (7)	9±2 (12)	4±2 (7)	12±3 (29)*+
LONG-RE	40±4 (22)	33±12 (2)	10±2 (10)	6±1 (11)	23±3 (45)+
AHP HALF-DECAY TIME (ms)					
NORMAL MG	22±1 (54)	19±2 (5)	25±1 (31)	49±3 (28)	28±1 (118)
NO-RE					30±1 (65)
LOW-RE					27±2 (38)
MED-RE	13±1 (3)	16±1 (9)	23±2 (12)	43±3 (8)	27±1 (32)
LONG-RE	20±1 (33)	23±1 (3)	24±2 (18)	44±3 (19)	27±1 (73)
AXONAL CONDUCTION VELOCITY (ms)					
NORMAL MG	97±1 (55)	100±4 (7)	99±2 (25)	81±2 (24)	94±1 (111)+
NO-RE					70±1 (72)*
LOW-RE					64±1 (38)*
MED-RE	85±2 (3)	82±1 (10)*	80±1 (14)*	75±1 (9)*	74±2 (36)*
LONG-RE	96±2 (33)	94±5 (3)	96±2 (18)	84±2 (19)	90±1 (73)+

a. Means ± SE (number of units).
b. * = Significant difference from NORMAL ($p<0.01$);
c. + = Significant difference from NO-RE ($p<0.01$);

There was no change in mean AHP half-decay time following axotomy (Gustafsson, 1979; Kuno et al. 1974b), but the range was compressed from both extremes (Fig. 4-2A). In contrast, Gustafsson and Pinter (1984b) reported a slight but significant increase in AHP duration for lumbosacral motoneurons (16 of which were triceps surae). Kuno et al. (1974a) also showed a slight increase in MG AHP durations following axotomy. This later study also examined the slow soleus motoneurons, which exhibited markedly decreased AHP durations following axotomy. This finding, in conjunction with the altered distribution of AHP durations, suggests that the difference between studies reflects the relative composition of fast and slow motor units in the motoneuron pool examined. The presence of approximately 25% slow units in MG may offset the change in AHP duration of fast units (Kuno et al. 1974a; Gustafsson and Pinter, 1984). Gustafsson and Pinter (1984) suggested that the compressed range for AHP duration with axotomy indicated that fast motoneurons increase AHP duration, while slow motoneurons decrease AHP duration. Alteration in AHP half-decay time could reflect changes in the kinetics of the Ca^{++}-dependent K^{+} conductance thought to underlie the AHP (Barrett et al. 1980; Krnjevick et al. 1978) or changes in the magnitude of membrane nonlinearities (Ito and Oshima, 1965; Gustafsson and Pinter, 1985).

Most studies on spinal motoneurons have reported an increase in input resistance with axotomy (Gustafsson, 1979; Gustafsson and Pinter 1984). In contrast, Kuno and Llinas (1970) reported no change of input resistance following nerve section. The discrepancy may be due in part, to different criteria for recognizing axotomized cells.

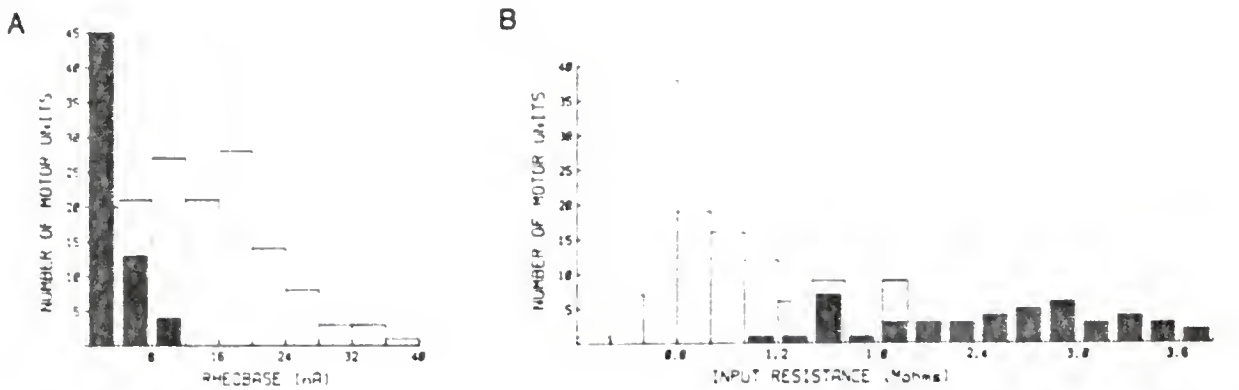

Figure 4-1. Frequency histograms for MG motoneuron properties following section of the MG nerve (filled), compared to normal MG motoneurons (unfilled). (A) Rheobase, note decreased range and mean vs. controls. (B) Input resistance, note similar range, but shifted to higher values than controls. (3) Ahp half-decay time, note range compressed from both extremes, no change in means. (D) axonal conduction velocity, range is decreased and mean is lower.

Kuno and Llinas (1970) included only those cells exhibiting a 'partial' active dendritic response to afferent input, in their axotomized population. Also, in that study motoneurons were axotomized by ventral root section, as opposed to peripheral nerve section in the present work (also Gustaffson, 1979; Gustafsson and Pinter, 1984). The axon reaction is known to be dependent upon the length of the intact proximal stump (Kuwada and Wine; 1981, Lieberman, 1971; Mendell et al. 1976). The range of values for input resistance was similar to that of normal MG, although shifted to higher values (Fig. 4-1B). Mechanisms thought responsible for alterations of input resistance with axotomy are discussed by Gustafsson and Pinter (1984).

Rheobase was decreased in axotomized motoneurons, in agreement with earlier reports (Eccles et al. 1958; Kuno and Llinas, 1970; and Gustafsson, 1979). The range of values seen for rheobase was compressed as well as shifted to lower values (Fig. 4-1A). Presumably the decreased rheobase indicates altered excitability of the initial segment and the soma-dendritic membrane (Eccles et al. 1958; Faber, 1984).

The relationship between rheobase and input resistance is altered by axotomy (Fig. 4-3A). The range of values was compressed. The mean value for rheobase/input resistance was lower than normal (Table 4-2).

When compared to motoneurons from normal type S units, axotomized MG motoneurons had significantly higher input resistance, slower axonal conduction velocity and lower rheobase/input resistance. Thus, while some axotomized cells fell within the type S range for some parameters, they were not identical in properties to normal type S motoneurons.

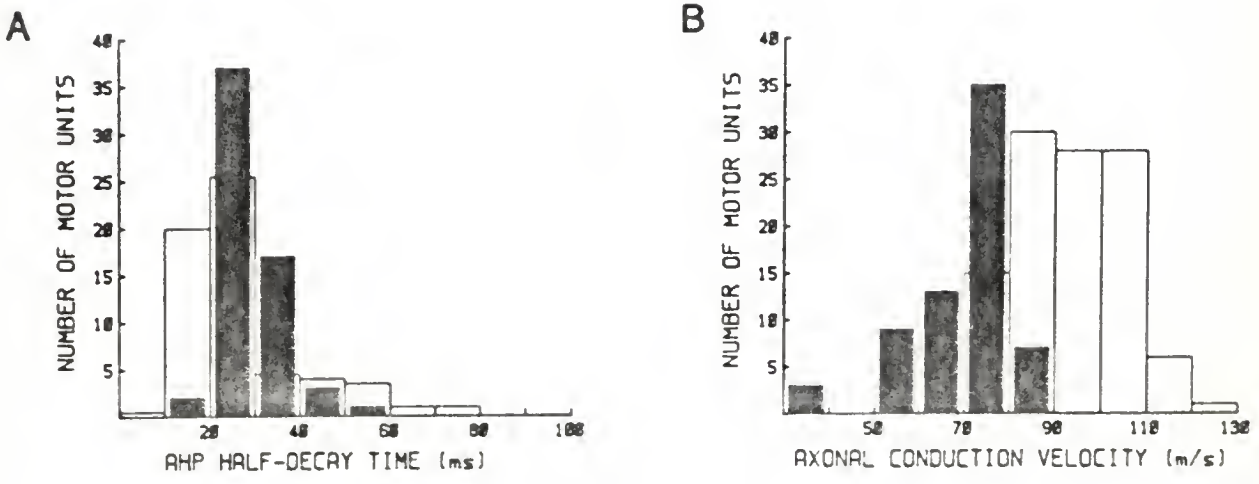

Figure 4-2. Frequency histograms for MG motoneuron properties following section of the MG nerve (filled), compared to normal MG motoneurons (unfilled). (A) AHP half-decay time, note range compressed from both extremes, no change in means. (B) axonal conduction velocity, range is decreased and mean is lower.

In the no-re population, the correlation seen in control motoneurons between rheobase and input resistance was lost (Fig. 4-3A), as was that between AHP half-decay time and axonal conduction velocity (Fig. 4-4A). There was no segregation of motoneurons with respect to these properties. For AHP half-decay time vs. axonal conduction velocity, 85% (51/60) of motoneurons sampled were outside the normal range for either fast or slow motoneurons. Motoneuron types based on the ratio rheobase: input resistance and AHP half-decay time (Zengel et al. 1985) could not be recognized in the no-re population.

These data are consistent with a 'dedifferentiation' of motoneuron properties from the normal adult state (Kuno et al. 1974a; Gustafsson and Pinter, 1984). While some properties of axotomized motoneurons are similar to those of normal type S motoneurons (rheobase, input resistance, rheobase/input resistance), AHP half-decay time is shorter, and axonal conduction velocity is slower than in normal type S units. Thus, the dedifferentiated state is not simply an 'S' or 'super S' motoneuron type (Gustafsson and Pinter, 1984).

Early Reinnervation

We examined two animals (39 and 40 days post-operative: low-re) which had MG muscles which generated 41 and 115 g-wt. twitch tension, respectively, in response to MG nerve stimulation. Previous studies indicate that a minimum of about four weeks is required before muscle contraction can be elicted by nerve stimulation (Eccles et al. 1962; Gordon and Stein, 1982b).

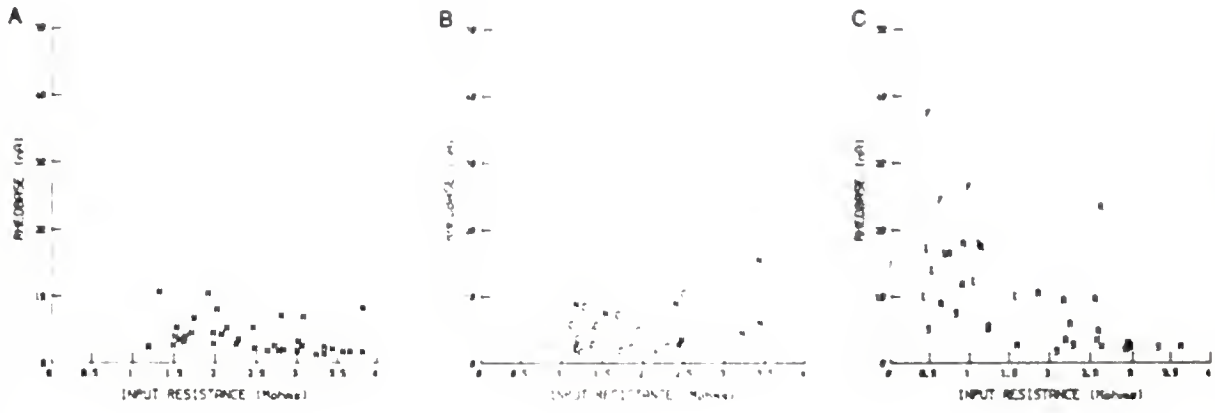

Figure 4-3. Relationships between rheobase and input resistance following section of the MG nerve, and at early and intermediate times of reinnervation. (A) axotomy, note uniformity of the sample and altered distribution (N= non-contract). (B) low-re, distribution is similar to no-re stage; no differences between contracts (C) and non-contracts (N). (C) med-re, note reestablishment of segregation by motor unit type, although distribution is not at normal levels.

Of 37 motoneurons sampled, 20 elicited muscle unit contraction (57%). Assuming 280 motoneurons in the normal MG motor nucleus (Boyd and Davey, 1968; Burke et al. 1977), this suggests that approximately 160 axons innervated MG muscle.

Motoneuron electrical properties. Low-re motoneurons had overall mean values which were significantly different from controls for all motoneuron electrical properties except AHP half-decay time (Table 4-2). In most cases the overall means were indistinguishable from the no-re population. There were no significant correlations between any motoneuron parameters at the low-re stage, unlike controls, but similar to axotomy (Figs. 4-3B, 4-4B).

Motoneurons at the low reinnervation stage were divided into two groups on the basis of whether they elicited muscle contraction (contracts) or not (non-contracts). The only differences in mean values between these two groups were rheobase and input resistance (both lower in contracts; Table 4-3). The similarity between properties of contracts and non-contracts could be interpreted in two ways: either making functional reconnection is not sufficient for re-establishment of normal motoneuron properties, or additional time is required following reinnervation for normal electrical properties to be expressed.

Kuno et al. (1974b) found two MG motoneurons (five months self-reinnervated muscle) which did not elicit muscle tension, but did not differ from connected cells in AHP duration, axonal conduction

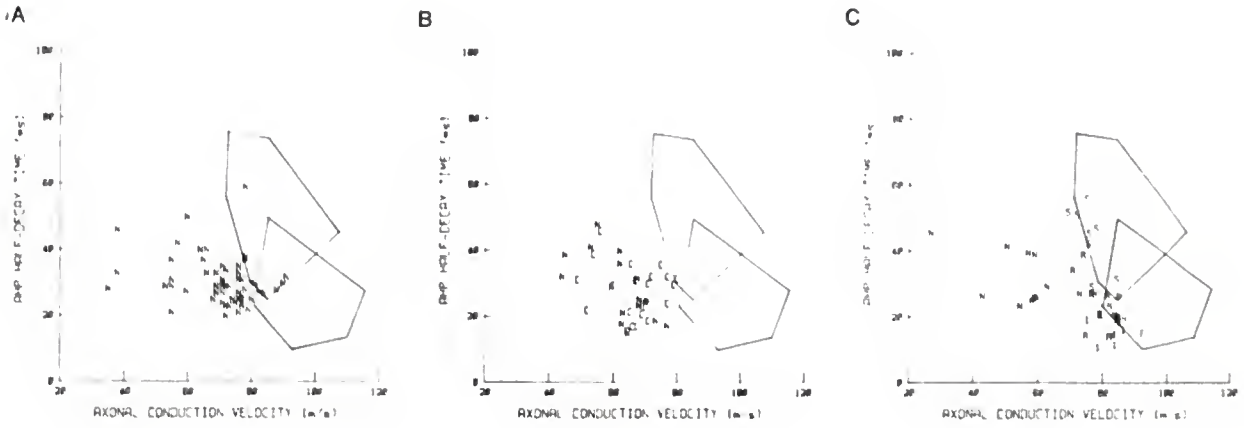

Figure 4-4. Relationships between AHP half-decay time and axonal conduction velocity after nerve section, and at early and intermediate stages of reinnervation. (A) no-re. Virtually all motoneurons fall outside the normal range. (B) low-re. Virtually all motoneurons fall outside the normal range. There was no difference between contracts (C) and non-contracts (N). (C) med-re. Many contracts (F,I,R,or S) approach or enter the range of control motoneurons, while all non-contracts (N) fall outside the normal range. There was segregation of fast and slow motoneurons. Solid lines in all graphs outline the distribution of slow (upper) and fast motoneurons in controls.

velocity, or action potential amplitude. They preferred the explanation that functional reconnection is neither necessary nor sufficient for recovery of motoneuron properties.

That functional reconnection is necessary for re-establishment of normal electrical properties is suggested by the similarity between non-contracts at all times following axotomy (Table 4-4). At the med-re and long-re stages non-contracts had mean values significantly different from the overall sample at the same stage. Mean values for rheobase and rheobase/input resistance (med-re only), input resistance, and axonal conduction velocity also differed from type S units at the same stage of recovery. AHP half-decay times were short, as in axotomy, and different from control type S units. These data are consistent with suggestions, based on recording extracellular action potentials of single units, that the effects of peripheral axotomy are not continuous, but reach a stable, plateau value (Davis et al. 1978). This suggests that motoneuron survival following peripheral axotomy is not dependent upon connection to muscle in regenerating adult motoneurons, although expression of normal electrical properties is. All cells at the long-re or med-re stages which made functional reconnection with the MG muscle showed recovery of motoneuron properties, suggesting that reconnection, with sufficient recovery time, is sufficient for expression of mature electrical properties.

There were some non-contracts in the long-re and med-re populations with properties in the normal range for one or several motoneuron properties. These cells could have been injured in the final dissection or perhaps represent alpha-fusimotor innervation (Gregory et al. 1982).

Table 4-3. LOW-RE Motoneuron Electrical Properties: Contract vs. Non-Contract. [a,c]

	CONTRACTS	NON-CONTRACTS
RHEOBASE (nA)	4±1 (20)*	6±1 (17)
RN [b] (Mohms)	1.5±0 (20)**	2.3±0 (15)
RHEOBASE/RN	3±0 (19)	3±1 (15)
AHP HALF-DECAY TIME (ms)	27±2 (20)	28±2 (17)
AXONAL C.V. [d] (m/s)	66±2 (20)	61±2 (17)

a. Means ± SE (number of cells).
b. RN = input resistance.
c. * = significant difference from NON-CONTRACT (alpha = 0.05); ** (alpha = 0.01).
d. C.V. = conduction velocity.

Table 4-4. Motoneuron Electrical Properties of Non-contracts: Self-reinnervation Model. [a,c]

	NO-RE	LOW-RE	MED-RE	LONG-RE
RHEOBASE (nA)	4±1 (67)	6±1 (17)*	3±0 (11)	7±1 (8)
RN [b] (Mohms)	3±0 (60)	2±0 (15)	3±0 (12)	2±0 (5)
RHEOBASE/RN	2±0 (59)	3±1 (15)	1±0 (11)	5±4 (5)*
AHP HALF-DECAY TIME (ms)	30±1 (65)	28±2 (17)	30±2 (12)	30±4 (8)
AXONAL C.V. [d] (m/s)	70±1 (72)	61±2 (17)	56±3 (12)*	64±7 (8)

a. Means ± SE (number of cells).
b. RN = Input resistance.
c. * = significant difference from NO-RE (alpha = 0.05); ** (alpha = 0.01).
d. C.V. = conduction velocity.

At the long-re stage the vast majority of non-contracts were outside of the normal range for AHP half-decay time vs. axonal conduction velocity (c.f. Fig. 3-4).

Muscle unit contractile properties. All speed-related properties of the whole muscle twitch were prolonged at the low-re stage (Table 4-1; twitch time-to-peak, half-relaxation time, half-rise time). Prolonged isometric time-to-peak has been reported to occur with denervation (Kean et al. 1974; Spector, 1984), with relaxation prolonged more than twitch rise-time. This has been attributed to alterations in the sarcoplasmic reticulum, troponins, and myosin in the denervated muscle fibers (Cecchi et al. 1984; Spector, 1984). At the low-re stage, motor units did not fit into adult motor unit types and so were treated as a single population. The mean value for low-re units' potentiated twitch time-to-peak was not significantly different from control (Table 4-5). Half-relaxation time was prolonged in low-re units. Amplitudes of potentiated twitch and maximum tetanus were greatly reduced in low-re units. All low-re units were fatiguable, but most showed residual tension at the end of the fatigue test.

We found no significant correlations between motor unit contractile properties in the low-re population. The relationship between twitch time-to-peak and maximum tetanus (-0.54, $p<0.07$)) approached significance.

These results are in agreement with Gordon and Stein (1982a,b) in that initially after reinnervation mean unit tensions were smaller, with no correlations between motor unit speed and tension.

Table 4-5. Muscle Unit Contractile Properties: Time Course of Self-Reinnervation.[1,2]

	FF	FI	FR	S	ALL
TWITCH TIME-TO-PEAK [d] (ms)					
NORMAL MG	29±1 (56)	28±2 (7)	26±1 (30)	58±4 (22)	34±2 (115)
LOW-RE					38±2 (12)
MED-RE	30±2 (3)	28±2 (10)	30±1 (14)	59±4 (9)	37±3 (36)
LONG-RE	28±1 (32)	25±5 (3)	27±1 (17)	55±3 (18)	34±2 (70)
TWITCH TENSION [d] (g-wt.)					
NORMAL MG	18±1 (56)	4±1 (7)	1±0 (28)	0.2±0 (22)	9±1 (113)
LOW-RE					0±0 (12)*
MED-RE	19±1 (3)	6±2 (10)	1±0 (13)	0.4±0 (9)	4±1 (35)*
LONG-RE	10±1 (32)*	2±6 (3)	1±0 (17)	1.0±0 (18)	5±1 (70)*
TWITCH HALF-RELAXATION TIME [d] (ms)					
NORMAL MG	24±1 (56)	27±2 (7)	26±1 (29)	74±8 (19)	33±2 (111)
LOW-RE					44±5 (12)
MED-RE	30±6 (3)	29±2 (10)	31±2 (13)	69±9 (9)	40±4 (35)
LONG-RE	28±2 (32)	24±5 (3)	25±2 (16)	51±4 (15)	32±2 (66)
MAXIMUM TETANIC TENSION (g-wt.)					
NORMAL MG	61±3 (84)	20±3 (7)	13±1 (45)	7±1 (39)	35±2 (175)
LOW-RE					4±1 (23)*
MED-RE	76±7 (3)	25±6 (10)	9±2 (14)	4±1 (9)	18±3 (36)*
LONG-RE	37±4 (31)*	20±0 (3)	13±3 (17)	6±1 (16)	23±2 (67)*
FATIGUE INDEX					
NORMAL MG	0.0±0 (82)	0.5±0 (7)	1.0±0 (45)	1.1±0 (40)	0.6±0 (174)
LOW-RE					0.2±0 (11)
MED-RE	0.1±0 (3)	0.5±0 (10)	0.9±0 (13)	1.0±0 (8)	0.7±0 (34)*
LONG-RE	0.1±0 (30)*	0.5±0 (3)	1.2±0 (17)	1.1±0 (13)	0.6±0 (63)

a. Means ± SE (number of units).
b. * = Significant difference from NORMAL MG ($p<0.01$).
c. Potentiated twitch.

Muscle histochemistry. Low-re MG contained 50% type FG muscle fibers, 28% type FOG, and 23% type SO, similar to controls (Table 4-7). It is uncertain to what extent this reflects the original innervation of the muscle fibers, as fiber type grouping (Dubowitz, 1967; Romanul and Van Der Meulen, 1967; Karpati and Engel, 1968a) was not seen at this stage. Mean muscle fiber area was decreased at the low-re stage, with fast fibers (types FG and FOG) affected more than type SO fibers (Table 4-7).

The muscles from the animals intermediate in recovery between low-re and med-re stages contained 55% type FG, 17% type FOG, and 28% type SO fibers (FE12) and 44% type FG, 32% type FOG, and 25% type SO fibers (FE17). Of 12 motor units sampled in cat FE12, six were type FF, one was type FR, three were type S motor units, and two were non-contracts. No motor unit data were obtained from FE17.

Relationships between motoneuron electrical properties and muscle unit contractile properties. We found no significant correlation between any combination of motoneuron and muscle properties at the low-re stage (Gordon and Stein, 1982b). At two months following the initial surgery, Gordon and Stein (1982a,b) reported no correlation between action potential amplitude (correlated with axonal conduction velocity) and twitch tension, or between twitch contraction time and twitch tension.

Medium Reinnervation Stage

Two animals were examined which generated greater than 50% of long-re whole muscle twitch tension (63 and 71 days; med-re). At this stage motor unit types could be distinguished by contractile criteria. The proportion of fast and slow units at the med-re stage was similar to normal MG (75% fast, 25% slow vs. 76:24 in normal MG; 69:31 in long-re MG). There were 8% type FF, 28% type FI, 39% type FR and 25% type S units (controls: 48:4:24:24; long-re: 50:4:15:31; Table 7). The increased proportion of type FI units appears to be at the expense of type FF units. This may indicate a general increase in fatigue resistance of fast units at this stage, perhaps due to previous polyneuronal innervation, increased recruitment due to lowered overall force-generating ability of the muscle, or a general increase in oxidative enzymes as a function of reinnervation.

Statistical differences between means for motor unit parameters at the med-re stage were similar to normal and long-re motor units (Table 4-6).

Motoneuron electrical properties. The overall means for med-re motoneuron electrical properties show a definite shift towards those of the long-re and normal populations (Table 4-2), although axonal conduction velocity, rheobase/input resistance and rheobase were still lower, and input resistance was still higher in med-re than long-re or normal motoneurons (Table 4-2). Mean AHP half-decay time was essentially the same at all stages.

Table 4-6. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types for MED-RE Motor Units.

	MED-RE	
		P
Axonal Conduction Velocity	NS	0.05
Rheobase	F>I>(R,S)	0.01
Input Resistance	NS	0.05
Rheobase/Input Resistance	F>(I,R,S)	0.01
AHP Half-Decay Time	NS	0.05
Twitch Tension [a,b]	F>I>(R,S)	0.01
Twitch Time-To-Peak [b]	(F,I,R)<S	0.01
Twitch HRT [b,c]	(F,I,R)<S	0.01
Maximum Tetanic Tension [a]	F>I>(R,S)	0.01

a. The same result was obtained for raw data and data normalized for body weight.
b. Potentiated twitch.
c. HRT = half-relaxation time.

Mean values by motor unit type, for motoneuron electrical properties, were generally similar to properties of the same type of motoneuron in normal or long-re MG motoneurons. Only input resistance (type FR) and axonal oonduction velocity (types FF and FR) differed between long-re and med-re motoneurons of the same type (Table 4-2).

Fig. 4-3C shows the relationship between rheobase and input resistance for med-re motoneurons. Note that segregation by motor unit type is re-established at this stage, although lower rheobases and higher input resistances tend to alter the distribution from normal. The correlation between log rheobase and input resistance was -0.80 ($p<0.0001$). Within unit types log rheobase and input resistance were correlated for type S units only (-0.80, $p<0.03$)).

AHP half-decay time was negatively correlated with axonal conduction velocity across all units ($r= -0.50$, $p<0.0001$; Fig. 4-4C), as in normal and long-re motoneurons (Fig. 3-2). This relationship was significant within type FR units (-0.79, $p<0.002$), but not type FF or type S units. There was some segregation into fast and slow groups by the relationship AHP half-decay time: axonal conduction velocity (Fig. 4-4B), although this relationship was not as in normal or long-re motoneurons (Fig 3-4). Five of eight type S motoneurons were within the normal range for this relationship (63%), as were 10 of 22 fast motoneurons (45%). All non-contracts (12/12) fell outside the normal range for either fast or slow motoneurons.

Motoneuron type was determined according to the criteria of Zengel et al. (1985; AHP half-decay time >30ms = fast, AHP <30ms = slow; type FF units have rheobase/input resistance >18, type S units <7, and type

FR units between 7 and 18). At the med-re stage, there was an 82% agreement (18/22) between electrically determined motoneuron type, and motor unit type determined from contractile properties. AHP half-decay time predicted fast and slow motor units in 91% of cases (29/32).

Muscle unit contractile properties. At this stage certain contractile properties have recovered more than others (Table 4-5). The overall means for twitch time-to-peak, half-rise time, and half-relaxation time were similar to normal. Twitch tension (raw data and normalized by body weight) recovered nearly to the level shown by long-re units (smaller than control values). This was also true of maximum tetanus (raw and normalized). Overall fatigue index was higher than for normal or long-re units, reflecting the increased proportion of types FR and FI units. All units displayed some residual tension after the fatigue test.

Across the whole population, twitch time-to-peak was significantly correlated with the log of twitch amplitude/body weight ($r= -0.32$, $p<0.04$) and tetanic tension/body weight ($r= -0.58$, $p<0.0001$), as in normal and long-re units (CHAPTER III). These relationships did not hold within any motor unit type.

Relationships between motoneuron electrical properties and muscle unit contractile properties. As noted above, there was good agreement between motoneuron type and motor unit type at the med-re stage. Most correlations found between motoneuron and muscle properties in normal and long self-reinnervated MG had recovered by the med-re stage. Across all units, AHP half-decay time was positively correlated with twitch time-to-peak (0.82, $p< 0.0001$). This relationship was not significant

within motor unit types. Axonal conduction velocity was correlated with twitch time-to-peak (-0.51, $p<0.0001$), the log of twitch tension/body weight (0.43, $p<0.01$), and log maximum unit tetanic tension/body weight (0.63, $p<0.0001$). The relationship with time-to-peak was only significant within FR units (-0.73, $p<0.03$) and the relationship with log tetanic tension/body weight within FR units (0.59, $p<0.02$). There were no significant correlations between axonal conduction velocity and log twitch tension/body weight within motor unit types.

Gordon and Stein (1982a,b) noted a parallel recovery of axonal action potential amplitude and motor unit twitch tension. In their study, units recovered normal tension five to six months following surgery (nerve-nerve suture). The normal positive correlation across the whole population of units, for action potential amplitude and twitch tension, was lost in early reinnervation, and did not recover until the sixth postoperative month (Gordon and Stein, 1982b). The normal negative correlation between twitch contraction time and tension recovered earlier, at three months. These authors felt that the early lack of correlation between axonal action potential amplitude and twitch tension may be explained by motor units being heterogeneous with respect to metabolic enzymes at early stages. They suggested that recovery of normal relationships between nerve and muscle depends upon time and extent of whole muscle recovery, with whole muscle recovery having the best predictive value.

Muscle histochemistry, muscle fiber areas, and innervation ratios. Histochemistry did not indicate the same magnitude of shift towards fatigue-resistant units as did the motor unit type distribution. At this

stage 43% of the muscle fibers were type FG, 24% type FOG, and 33% type SO (Table 4-7). The med-re stage was the first one in which fiber-type grouping (Dubowitz, 1967) of muscle fibers was seen. The overall distribution was significantly different from controls (Chi-square, 95% confidence level). Comparison to expectations for an assumed hypergeometric distribution showed no significant differences for any individual muscle fiber type. Oxidative fibers (FOG+SO) made up 57% of the population, as compared to 44% in controls (31% in long self-reinnervated). This change is in the direction suggested by the motor unit type distribution. Perhaps the qualitative comparison of NADH staining intensity is insufficiently sensitive to detect the degree of changes in enzyme levels, or the relationship between NADH staining and fatigue resistance is not particularly strong (Baldwin et al. 1984).

We estimate physiological cross-section at 2.4cm^2. Mean fiber areas and relative innervation ratios are found in Table 4-7. Mean fiber areas show recovery towards normal values for types FG and FOG fibers, while type SO fibers are smaller than controls. (see CHAPTER III).

With denervation, types FG and FOG fibers atrophy to a greater extent than type SO fibers (low-re stage; see also Lowrie and Vrbova, 1984). With time, type SO muscle fibers decrease in size until the med-re stage, then recover to normal levels. Type FOG fibers recover to normal size earlier, at the med-re stage. Type FG fibers, on the other hand, never recover to control levels, although type FG fibers remain the largest. Motor unit tension recovery follows the pattern of recovery of muscle fiber area (see below).

Table 4-7. Motor Unit Types, Muscle Fiber Types, and Innervation Ratios: Time Course of Self-Reinnervation. [e]

MUSCLE FIBER TYPES

% Histochemical Composition

	FG	FOG	SO	n/N [a]
NORMAL MG	56	23	21	4200/7
LOW-RE	50	28	23	900/2
MED-RE	43	24	33	900/2
LONG-RE	54	15	31	3000/5

Observed Mean Fiber Area (um^2)

				ALL
NORMAL MG	3873	2264	1972	3104
LOW-RE	1439	1274	1803	1491
MED-RE	2125	1634	1211	1714
LONG-RE	3098	2355	2296	2738

EQUIVALENT MOTOR UNIT TYPE

% Of Population

	FF+FI	FR	S	n/N [a]
NORMAL MG	48+4	24	24	176/16
MED-RE	8+28	39	25	36/2
LONG-RE	46+4	24	26	72/4

Est. Number in Pool [d]

				ALL [b]
NORMAL MG	134+11	67	67	280
LOW-RE	X	X	X	160
MED-RE	2+59	82	53	210
LONG-RE	116+10	61	66	252

CALCULATED VALUES

Relative IR [c]	FF+FI	FR	S
NORMAL MG	1.1	1.0	0.9
MED-RE	1.2	0.6	1.3
LONG-RE	1.1	0.6	1.2

a. Number of Cells/Number of Animals.
b. [280 - (280)(% non-contracts)]
c. Relative Innervation Ratio = %Muscle Fiber Type/% Motor Unit Type.
d. Estimated as (Total number of motoneurons)(% motor unit type).
e. Motor unit types could not be distinguished at the LOW-RE stage.

Relative innervation ratios indicate a possible disadvantage of type FR motoneurons and advantage of type S motoneurons in capturing muscle fibers. At the long-re stage there is an even greater trend towards low relative innervation ratio of type FR units and increased ratio in type S units than observed at the long-re stage (CHAPTER III).

Gordon and Stein (1982a,b) used chronic recording techniques to follow the time course of self-reinnervation of cat MG. They calculated that the estimated number of units did not change from early reinnervation to the plateau of recovery although whole muscle and single unit tensions increased (see also Kuno et al. 1974a). These authors speculated that the primary change was an increase in fiber diameter. Our data support the notion that a large proportion of the increase in muscle tension from the med-re stage to the long self-reinnervated stage is due to increase in fiber area (160% increase in mean fiber area, 120% increase in whole muscle twitch tension, 129% increase in mean motor unit tetanic tension). After nine months reinnervation, all three of these parameters were still below normal values (88%, 78%, 63% of normal, respectively).

We saw a larger proportion of non-contracts at the med-re stage, relative to nine-month reinnervated MG (25% vs. 9%). We estimated the number of innervating axons from the percentage of non-contracts (12/48 = 25%) as 210. This indicates that a proportion of axons, which eventually form functional connections with muscle fibers, had not yet done so at 63 or 71 days. Alternatively, a greater percentage of units at this stage which are connected do not elicit measurable levels of tension.

At the med-re stage we found significant correlations between contractile parameters, as well as between axonal conduction velocity or AHP half-decay time and contractile parameters. It appeared however, that some axons destined to do so had not made functional connections at this time (63-71 days). In general, recovery of muscle and motor unit properties occurred in parallel.

Discussion

This study examined the influence of functional connection with muscle on expression of motoneuron electrical properties. Also examined was the time course of recovery of motor unit properties during self-reinnervation of the cat MG muscle. The purpose was to determine the level from which motoneurons must recover, and the progression of recovery, in order to place the long-term reinnervation results in perspective. In addition, such data were required for interpretation of ongoing studies of reinnervation of foreign muscles by motoneurons.

Following nine months recovery from nerve section and self-reunion, most motoneuron and muscle unit properties recover to control levels, and normal relationships between motoneuron and muscle properties are re-established (Bagust *et al.* 1981; Lewis *et al.* 1982; Chan *et al.* 1982; Burke, 1980; CHAPTER III). In this study we examined motoneuron electrical properties and the contractile properties of the respective muscle units, after intermediate periods of recovery from nerve section.

Following section of the MG nerve, MG motoneurons become more excitable to somatic current injection (decreased rheobase), depolarize more in response to a given current input (increased input resistance), and MG axons conduct impulses at a lower rate. Axotomized motoneurons

did not segregate on the basis of either the ratio rheobase: input resistance or the relationship of AHP half-decay time and axonal conduction velocity. All of these data support the notion that following axotomy, motoneurons 'dedifferentiate' (Kuno et al. 1974a; Gustafsson and Pinter, 1984). Dedifferentiation can be regarded as a shift to a growth state for the motoneuron (Watson, 1976).

Huiszar et al. (1975) noted the resemblence between axotomized motoneurons and those of kittens, and suggested that this dedifferentiation returns adult motoneurons to a state similar to early ontogeny. Gustafsson and Pinter (1984a) suggested that axotomized motoneurons dedifferentiate to a level similar to that of adult slow motor units. The present data show that while many electrical properties of axotomized motoneurons overlap the range of type S motoneurons, in most cases the mean values for axotomy are different, and certain parameters (AHP half-decay time, axonal conduction velocity) are very different in axotomized motoneurons than in normal type S motoneurons. In addition, the relationship between AHP half-decay time and axonal conduction velocity was different for axotomized motoneurons than for type S motoneurons. Thus type S motoneurons, as well as fast motoneurons, represent a differentiatiated state from axotomized or immature motoneurons.

Functional connection to muscle and expression of motoneuron electrical properties.

One issue we addressed was whether functional reconnection of motoneuron to muscle fibers was necessary or sufficient for recovery of

motoneuron electrical properties. Kuno et al. (1974b) measured axonal conduction velocity, action potential overshoot amplitude, resting membrane potential, and duration of the AHP at various times (up to five months) following self-reinnervation of cat MG and soleus. At early times, with incomplete reinnervation, they reported no difference between properties of those motoneurons which elicted muscular contraction, and those that did not (both groups appeared axotomized). They interpreted this as evidence that functional reconnection is not sufficient for recovery of normal motoneuron properties. Kuno et al. (1974b) reported two of 25 MG motoneurons which did not elicit muscle tension following five months recovery, but had AHP duration and axonal conduction velocity in the normal range. Their interpretation was that functional reconnection was not necessary for recovery of motoneuron properties. One potential complication bearing on this interpretation is that properties of axotomized motoneurons overlap somewhat with normal type S motor units in terms of electrical properties (Gustafsson and Pinter, 1984; this study). In the Kuno et al. (1974b) study, all MG motoneurons were regarded as 'fast', and all soleus motoneurons as 'slow'. Normal MG contains approximately 25% type S motor units (Burke et al. 1973; Burke, 1981; Fleshman et al. 1981; CHAPTER III).

Our data show that motoneurons which do not make functional reconnection with muscle display membrane electrical properties similar to axotomized motoneurons. At the low-re stage, electrical properties of motoneurons with functional reconnections, and those of non-contracts were not different from one another, and neither differed from the axotomized condition, in agreement with Kuno et al. (1974b). This could

indicate that functional reinnervation is not sufficient for recovery of motoneuron electrical properties or that time is required for these parameters to reach levels permitted by reconnection.

At the med-re and long-re stages, motoneurons without functional reconnection to muscle (non-contracts) have electrical properties different from those motoneurons which elicit muscle contraction. In particular, the relationships between rheobase and input resistance, and between AHP half-decay time and axonal conduction velocity, are different in these cells. At the long-re stage, all motoneurons with functional connections had electrical properties similar to normal MG motoneurons innervating the same type of motor unit. At the med-re stage, motoneuron properties were not fully recovered, but all cells which elicited muscular contraction showed electrical properties altered significantly from axotomy (in the direction of controls), and segregated according to motor unit type with respect to rheobase, input resistance, and AHP half-decay time. Collectively, these data suggest that functional reconnection is necessary for re-establishment of motoneuron electrical properties. In agreement with our results are those of Gordon and Stein (1982b) who reported a subpopulation of axons which did not elicit muscle contraction, and which did not recover normal action potential amplitude (extracellular; a function of axonal diameter; see also Gordon, 1983; Gutman and Sanders, 1943). All axons eliciting muscle contraction in their study recovered to normal levels of action potential amplitude by nine months to one year post-operatively.

Thus, our data suggest that functional reconnection is necessary and perhaps a sufficient condition for recovery of motoneuron electrical properties. A similar situation occurs in the goldfish Mauthner cell, where membrane electrical properties do not recover in the absence of connection to an end organ (Faber, 1984). In contrast, Kuwada and Wine (1981) found that the effects of axotomy on somatic excitability of crayfish central neurons was a transient one, regardless of whether the axon regenerated and formed new connections. There appears to be variability in the degree to which the periphery influences neural properties (Gordon, 1983).

Price (1974) suggested that the motoneuron soma receives two types of signal from its periphery, an axon-to-soma signal concerning the state of the axon, and a muscle-to-nerve-to-soma signal, dealing with the relationship between muscle and nerve. Consistent with this, many changes seen with axotomy in sympathetic ganglion cells can be induced by blockage of axonal transport by colchicine (Purves, 1976). Recent evidence suggests that muscle-derived trophic factors exist (reviewed in Slack et al. 1983), and that uptake of chemical signals from muscle is activity-dependent (Watson, 1969; Brown and Ironton, 1977; Duchen and Strich, 1968; Czeh et al. 1978). Block of action potential conduction with tetrodotoxin (TTX) has been shown to alter AHP duration in a way similar to axotomy in cat soleus motoneurons (Czeh et al. 1978), as well as AHP half-decay time, axonal conduction velocity, rheobase, and input resistance in cat MG motoneurons (Munson et al. 1985). Electrical stimulation distal to, but not proximal to the TTX cuff was observed to reverse the change in AHP duration in soleus motoneurons

(Czeh *et al.* 1978). Further experiments (Gallego *et al.* 1979) suggest that the metabolic state of the innervated muscle is the important variable, rather than activity *per se*. Thus the muscle exerts influence on the expression of motoneuron electrical properties.

Recognition of motor unit types and recovery of properties.

A second goal was to determine at what stage motor unit types are first recognizable, and how this relates to the level of recovery of motoneuron properties. We found that motor unit types became recognizable using contractile criteria at the med-re stage (8-10 weeks), before mean motoneuron electrical properties and mean muscle unit contractile properties had reached mature levels.

Although mean values for motoneuron electrical properties were not at normal levels, motoneurons segregated into types with respect to rheobase, input resistance, and AHP half-decay time at the med-re stage. At this stage most axons had made functional connections with muscle fibers, although apparently some axons destined to make connections had not yet done so. Relationships between motoneuron properties, between muscle unit speed and tension, and between motoneuron and contractile parameters, were relatively normal at the med-re stage.

Most of the increase in muscle and motor unit tension beyond the med-re stage can be accounted for by increase in muscle fiber area, although there may be a small increase in number of axons with functional reconnection. Motor unit tension recovery follows the pattern of muscle fiber area recovery. Muscle fiber area increased 160% from the med-re to the long-re stages, whole muscle twitch tension increased 120%, and mean motor unit tetanic tension increased 129%. Type FG muscle

fibers did not fully recover cross-sectional area, thus type FF motor unit tetanic tension was reduced. This failure of recovery could reflect altered activity (Lapointe and Gardiner, 1984; Spector, 1984), altered trophic relationships between nerve and muscle (Spector, 1984; Lapointe and Gardiner, 1984; but see Cangiano and Lutzemberger, 1980), or a mismatch between motoneuron firing rate and the muscle's ability to respond (Lowrie and Vrbova, 1984). In general, these data confirm the suggestions made by Gordon and Stein (1982b) for self-reinnervation of cat MG.

At the med-re stage a greater than normal proportion of the fast units were fatigue-resistant, as indicated by the reduced proportion of type FF, and increased frequency of types FI and FR motor units. It is likely that some motoneurons, which originally innervated type FF units, innervated more fatigue-resistant types FI and FR units at this stage. Lowrie and Vrbova (1984) reported increased fatigue resistance in rat muscle after crush injury to the nerve. Perhaps the increased resistance to fatigue reflects increased activation of type FF units due to the lowered tension of all motor units (see also Baldwin et al. 1984).

Increased fatigue resistance could also reflect increased activation during the stage of poly-neuronal innervation seen with reinnervation (McArdle, 1975; Letinsky et al. 1976; Rothshenker and McMahan, 1976), or be a general feature of the metabolism of differentiating muscle. Increased fatigue resistance of fast muscle is also a transient phenomenon in developing rat muscle (Lowrie and Vrbova, 1984). By nine months, fatigue resistance of self-regenerated cat MG units was as in normal MG (CHAPTER III; Chan et al. 1982; Burke, 1980).

There were no correlations between muscle tension and speed, axonal conduction velocity and twitch time-to-peak, or axonal conduction velocity and muscle tension at the low-re stage, but all these relationships had recovered by the med-re stage, when mean whole muscle tension was about 60% of that at nine months self-reinnervation. Gordon and Stein (1982b) reported that the normal relationship between unit contractile speed and twitch tension was lost in newly reinnervated MG, but recovered when whole muscle tension was about 50% of that eventually attained (3 months post-operative). In their study the relationship between tension and axonal action potential amplitude recovered more slowly (six months).

Gordon and Stein (1982b) suggested that the initial lack of correlations was due to the early-reinnervated units being heterogeneous in nature, since axons do not return to their original muscle fibers (Karpati and Engel, 1968; Kugleberg et al. 1970). In support of this notion, we find that recovery of relationships between motoneuron and muscle properties is coincident with the re-establishment of recognizable motor unit types. Also, muscle fiber type grouping was first evident at the med-re stage, suggesting that motoneurons had induced myosin and mitochondrial enzyme properties of their muscle unit by that time. Motoneuron properties and muscle unit properties were re-established with a similar time course.

Relative reinnervation of motor unit types. A third question was whether any motor unit type might be favored in the process of self-reinnervation of a mixed muscle. From the earliest stage that motor unit types could be recognized (med-re), there was no difference in the

relative proportions of fast (FF+FI+FR) vs. slow (S) motor units, suggesting no advantage for survival of particular motoneuron types.

We found a slight increase in the proportion of type SO muscle fibers at the med-re stage. Together with the unchanged proportion of type S motor units, this leads to an estimate of increased mean innervation ratio for type S units. This appeared to be at the expense of type FR units (type FOG muscle fibers). We observed a similar situation after nine months self-reinnervation (CHAPTER III). Since motor unit types could not be recognized before the med-re stage, it is not possible to determine whether this slight advantage was due to different rates of axonal growth (e.g. type S axons reaching the muscle first) or more successful competition after contacts were made. These data could be evidence for a slight advantage of type S motoneurons in competition for muscle fibers during reinnervation as suggested by Lewis et al. (1982; see also Ip and Vrbova, 1984).

Thus there is no strong evidence for an advantage in reinnervation by any motor unit type, although type S units may be more effective in competing for muscle fibers (see also CHAPTER III).

Comparison with ontogeny. There were some parallels between the recovery of motor unit properties following self-reinnervation, and the progression of normal ontogeny. The above mentioned transient phase of high fatigue resistance was one similarity (Lowrie and Vrbova, 1984). The resemblence between axotomized adult motoneurons and motoneurons of young kittens with respect to action potential overshoot, AHP duration, and axonal conduction velocity, as well as the relationship between AHP duration and conduction velocity led Huiszar et al. (1975) to suggest

that axotomy results in a dedifferentiation of motoneuron properties to a level similar to early development.

Most motoneuron and motor unit properties that have been investigated in kittens differentiate to the adult pattern by the sixth to tenth post-natal weeks (Kellerth et al. 1971; Bagust et al. 1973; Hammarberg and Kellerth, 1975; Hammarberg, 1974; Nystrom, 1968). This corresponds to the time at which kittens acquire the ability to run, and to the time of weaning (5-6 weeks; Peters, 1984).

During ontogeny, motoneuron soma size (Sato et al. 1977; Mellstrom and Skoglund, 1969), dendritic morphology (Scheibel and Scheibel, 1970), and axon myelinization (Berthold et al. 1983) continue to mature well beyond this 6-10 week period. Relative to these morphological changes, axonal conduction velocity does not reach adult values until late in development (although fast vs slow axons are differentiated from birth, or close to that time; Ridge, 1967; Huiszar et al. 1975; Bagust et al. 1973). Also, although the difference between AHP duration of fast and slow motoneurons is established by ten weeks, adult values are not attained until late in development (Hammarberg and Kellerth, 1975), perhaps related to the increase in dendritic tree development. This is especially true for the slow, soleus motoneurons (Huiszar et al. 1975).

Following axotomy, motoneuron properties dedifferentiate. If reinnervation of muscle is allowed, motor unit types are again recognizable by 8-10 weeks. Motoneuron and muscle unit properties mature in parallel, not reaching control levels until long after motor unit types are recognizable. The pattern of recovery of motoneuron electrical properties is shown in Fig. 4-5.

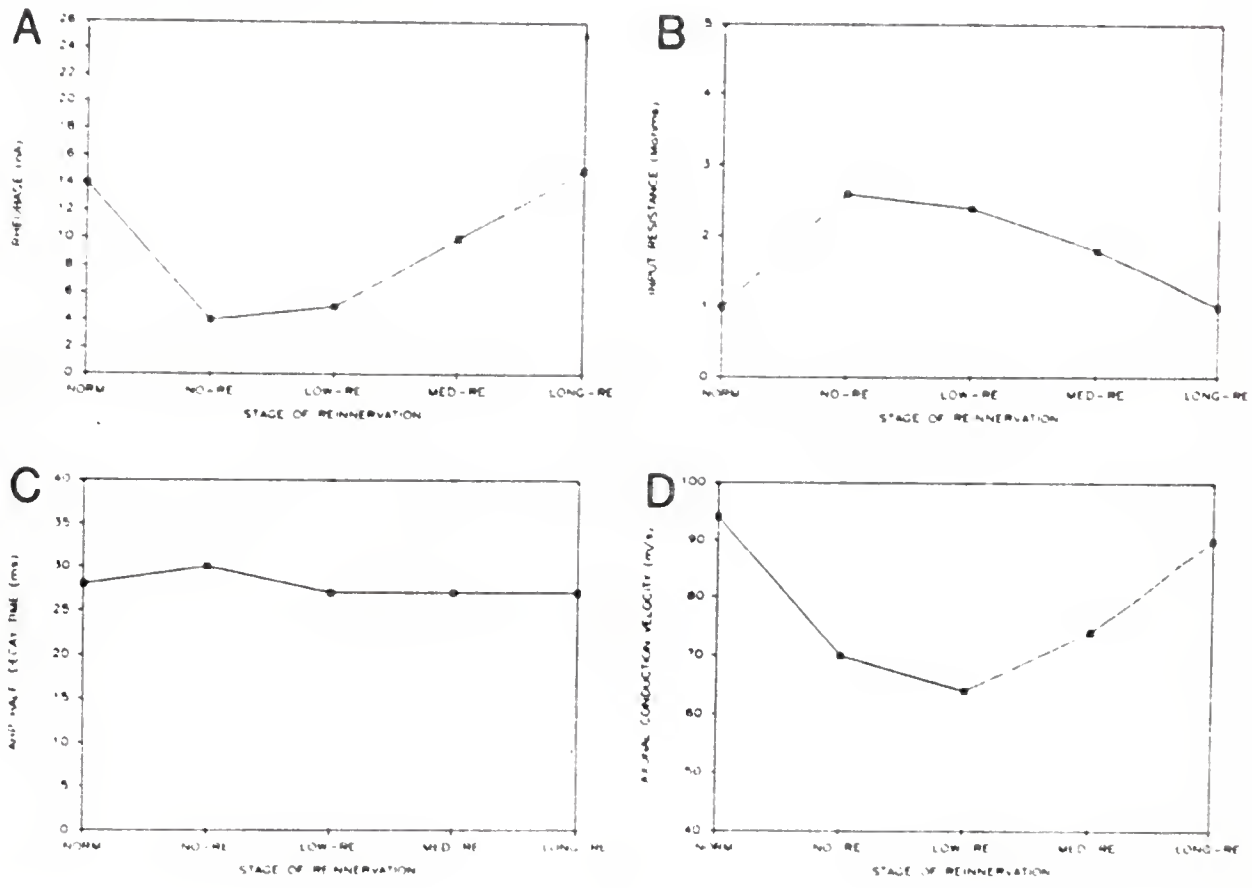

Figure 4-5. The time course of recovery of overall means for motoneuron electrical properties. (A) Rheobase decreases with axotomy, then gradulaly recovers to normal levels with time after reinnervation. (B) Input resistance increases with axotomy, then gradually recovers to normal levels with time after reinnervation. (C) AHP half-decay time. The mean values do not change with axotomy of reinnervation. (D) Axonal conduction velocity decreases with axotomy, then gradually recovers to control levels by nine-months post-operative.

Huiszar et al. (1975) found no clear relationship between alteration of particular motoneuron properties and changes in whole muscle twitch parameters, during postnatal development in cat MG and soleus. Similarly, we found no clear relationship between alteration of single motoneuron properties and changes in muscle unit properties during self-reinnervation of MG in adult cats. Rather, the overall motoneuron character (as indicated by motoneuron type) was strongly related to muscle unit type.

While there were many parallels with ontogeny, self-reinnervation of adult muscle is not a strict recapitulation of events occurring in development. In regeneration there are additional connective tissue barriers to axonal growth, and cues for axonal guidance are likely to be different (reviewed in Gordon, 1983; Vrbova et al. 1978). For example the basal lamina adjacent to the former endplate region is known to attract regenerating axons (Sanes, 1983). In regenerating adult muscles all muscle fibers are present before axonal ingrowth, and these muscle fibers have already reached a state of differentiation which was lost to an uncertain degree following loss of innervation. Although the results of reinnervation studies such as this one suggest that muscle fibers possess considerable plasticity, it is not certain whether this ability to alter properties is complete. The increased proportion of type SO muscle fibers following reinnervation could represent a type difference in muscle fiber or motoneuron plasticity.

CHAPTER V
PROPERTIES OF NORMAL LG AND SOLEUS

Introduction

The cat lateral gastrocnemius (LG) muscle has been an important model in studies of anatomical and histochemical compartmentation of muscle (English and Letbetter, 1982a,b; English and Weeks, 1984; Weeks and English, 1983, 1985), sensory partitioning within muscle (Van den Noven *et al.* 1983), differential usage of muscle compartments (English, 1984; Russel *et al.*1984; Rushmer *et al.* 1984), and motor reinnervation of muscle (Gordon and Stein, 1982a). Despite its importance, there are no detailed reports of motor unit contractile properties or distribution of motor unit types in this muscle, and even less information about motoneuron electrical properties. A few LG units are included in studies by Burke (1967; 14 units), Burke *et al.*(1973; 12 units), Burke *et al.* (1982; 12 units), Pinter *et al.* (1983, unknown number of units) and Hammarberg and Kellerth (1974; 36 units). These motor units appeared similar to those in medial gastrocnemius (MG), and were combined with data for that muscle in these reports. Gordon and Stein (1982a) present data for motor unit type distribution and unit tetanic tension from LG and soleus units combined. Hammant (1977) studied axonal conduction velocity and contractile properties for 76 LG units, and suggested that LG may differ from MG in possesing a higher proportion of fatiguable units, fewer type S units, and smaller tetanic tensions.

For interpretation of experiments investigating the plasticity of relationships between motoneuron electrical properties and muscle unit contractile properties (e.g. CHAPTERS VI, VII), it was important to know the proportions and properties of different motor unit types in LG, as well as the relationships between motoneuron and muscle unit properties in that muscle. To that end, LG whole muscle and single unit contractile properties, motoneuron electrical properties and their interrelationships were investigated in cat LG. In addition, data are presented for the same parameters in the soleus muscle, and these data are related to properties of MG and LG type S units. Previous studies (reviewed in Burke, 1981) have suggested that soleus units may differ from type S units in the gastrocnemii. Such information is important for interpretation of studies of neural influence upon soleus muscle properties. The criteria developed from MG motor units to categorize motoneurons into types based upon motoneuron electrical properties (Zengel et al. 1985) are shown to predict motor unit types accurately in cat LG and soleus as well.

Results

Whole Muscles

Table 5-1 presents data for whole muscle weight and contractile properties for normal LG, MG, and soleus muscles. LG was similar to MG in wet weight (Sacks and Roy, 1982) as well as all contractile properties. Soleus was smaller, slower (prolonged time-to-peak and

Table 5-1. Whole Muscle Properties: Normal MG, LG, and Soleus.[a]

	MG	LG	SOLEUS
Twitch Time+To+Peak[b] (ms)	32±1	32±1	75±7
Twitch Half+Rise Time[b] (ms)	11±0	10±1	19±2
Twitch Half+Relaxation Time[b] (ms)	22±2	23±2	88±6
Twitch Tension[b] (g+wt.)	2123±151	2089±142	688±51
Muscle Weight (g)	9.0±1	8.2±1	3.3±0
Muscle Wt./Cat Wt. (g/kg)	3.0±0	2.8±0	1.1±0
Twitch Tension/Cat Weight (g+wt./kg)	742±56	734±62	232±17
Twitch/Muscle Weight (g+wt./kg) (estimate of specific tension)	2.5±0	2.9±0	2.5±0

a. Means±SE (for MG, n= 14; for LG and SOLEUS, n= 15).
b. Twitch at muscle length at which maximum tension was obtained.

Table 5-2. Percent Muscle Fiber Types By Innervation Compartment: Normal LG.[a]

	FG	FOG	SO	n/N[b]
LG_m	87%	8%	5%	2718/3
LG_a	74%	9%	17%	3085/3
LG_b	61%	20%	19%	2730/3
LG_c	59%	18%	24%	3084/3

a) As defined by English and Letbetter (1982a,b).
b) Number of muscle fibers/number of animals.

half-relaxation times for twitch), and generated less tension than either gastrocnemius muscle (Murphy and Beardsley, 1974; Spector et al. 1980; Gardiner et al. 1978). Since these three muscles differ in architecture and mechanics, statistical analysis of differences in their contractile properties has little value.

Lateral Gastrocnemius

The LG muscle contained 63% type FF motor units, 5% type FI, 18% type FR, and 15% type S (n = 103; Table 5-2). This distribution was similar to MG, although there tended to be more fatiguable (FF+FI) units, and fewer type S units in LG than in MG (Hammant, 1977). Gordon and Stein (1982a) found 43% type FF, 24% type FR, and 33% type S units (n=92) for the combined LG + soleus population (no type FI units were reported). Our combined LG-S sample was 45% type FF, 3% type FI, 13% type FR, and 39% type S (n=143).

Overall, LG contained 65% type FG muscle fibers, 16% FOG, and 18% SO, (Table 5-2), in close agreement with the motor unit type distribution, and with previous reports of LG muscle histochemistry (Ariano et al. 1973; English and Letbetter, 1982b; Weeks and English, 1985). English and Letbetter (1982a,b) have shown that LG contains discrete subvolumes, or compartments, defined by innervation by separate nerve branches. Table 5-2 shows data for the various compartments individually. The overall distribution presented above was calculated by weighting the individual compartment distributions by the proportion of the motoneuron pool which innervates that compartment (12%, 19%, 34%,

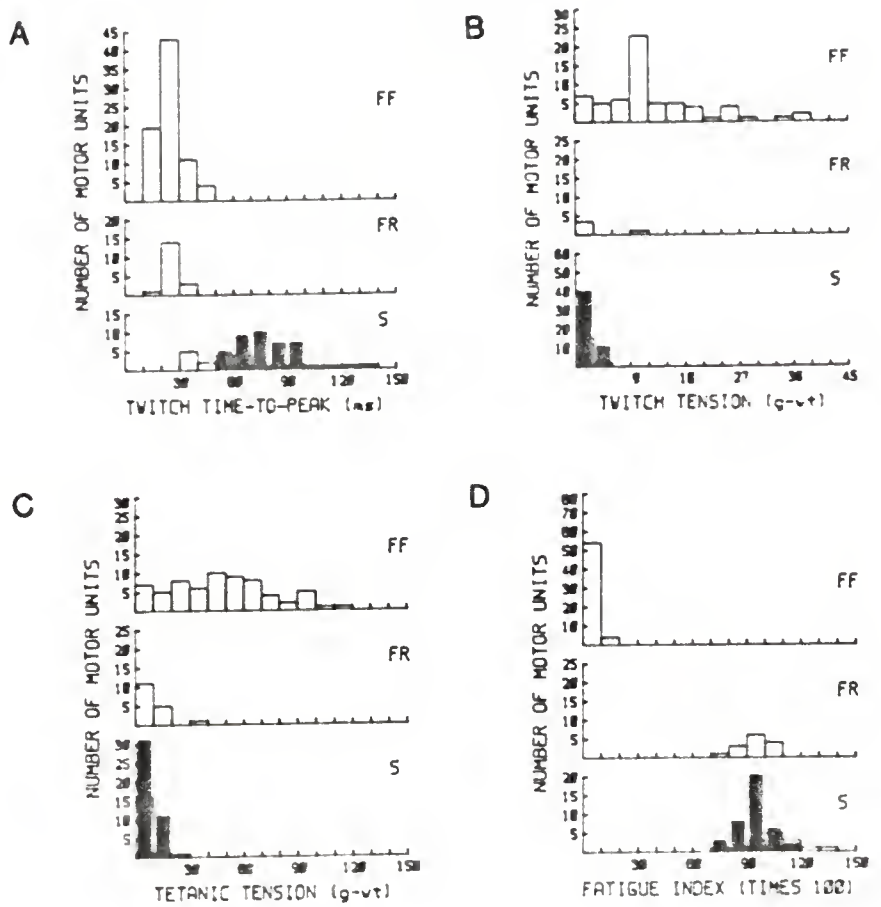

Figure 5-1. Frequency histograms for normal LG (unfilled) and soleus (filled) muscle unit contractile properties. (A) potentiated twitch amplitude (B) potentiated twitch time-to-peak (C) Tetanic tension (D) fatigue index. See text for explanation.

and 35% for LGm, LG1, LG2 and LG3, respectively; Weeks and English, 1985). These data are in close agreement with English and Letbetter (1982b).

The locations of 31 muscle units (5 animals) are shown in Fig. 5-2. This pattern is generally in agreement with histochemistry of individual compartments. Type S units were most frequent in the distal, medial (LG3) portion of the muscle; the proximal and medial region (LGm) was primarily type FF; and the lateral portions of the muscle had intermediate composition.

Lateral gastrocnemius muscle unit contractile properties. Mean values for LG muscle unit speed, tension, and fatiguability are very similar to MG, with only overall twitch/tetanus ratio statistically different ($p<0.01$; Table 3). Hammant (1977) found that mean maximum tetanic tension of LG type FF units was smaller than for MG type FF units. We found a similar tendency, although significant at the $p< 0.05$ level only. Slight variations in overall means reflect the slightly different motor unit type distributions. Frequency distributions for contractile properties are also similar to MG (Fig. 5-1; c.f. Fig. 3-1), although LG may contain more fatiguable units (Hammant, 1977). In addition, while the largest units in LG were of similar size to those in MG, a greater number of LG type FF motor units had low tetanic (<30 g-wt.; Fig. 5-1C) and twitch tensions (<9 g-wt.; Fig. 5-1B).

Across all units, there was a weak negative correlation between twitch time-to-peak and the log of twitch tension/body weight ($r=-0.36$, $p< 0.003$), and between time-to-peak and log of maximum tetanus/body

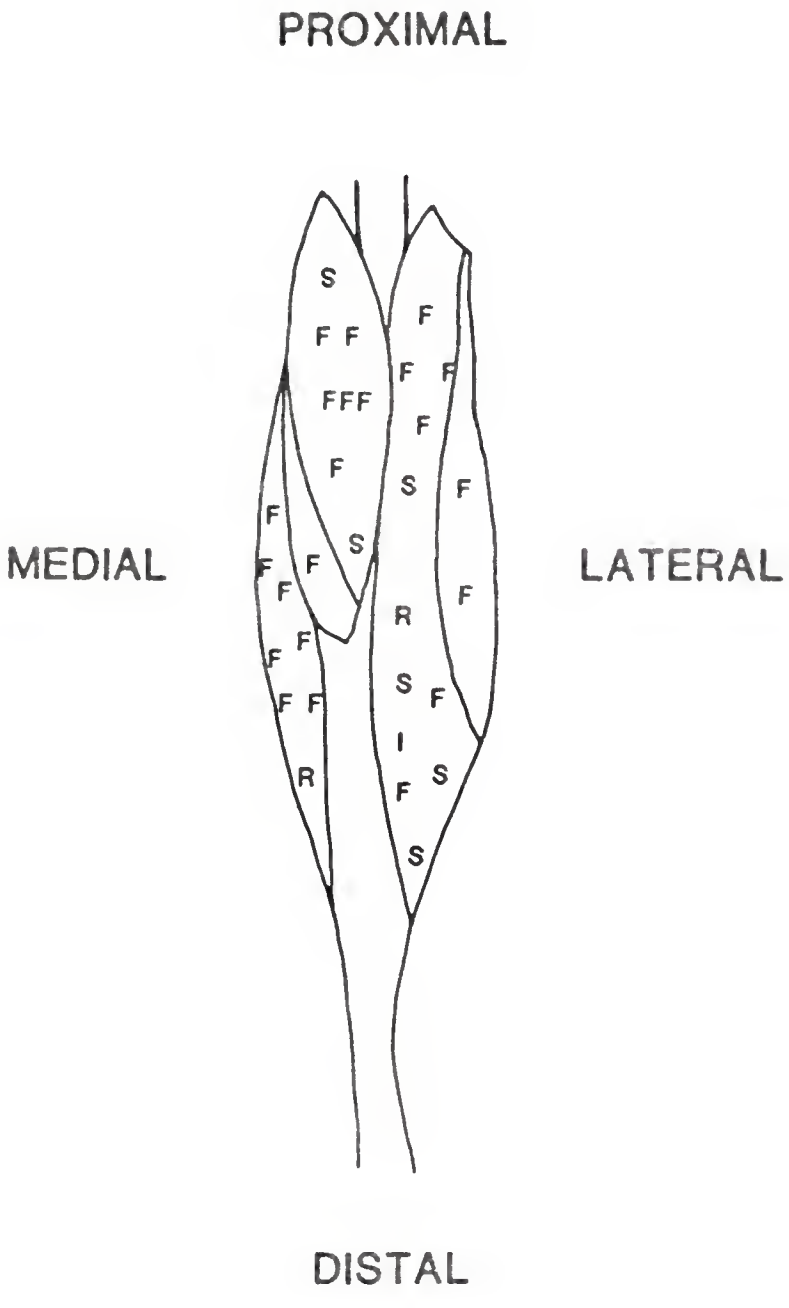

Figure 5-2. Location of 31 motor units (five cats) in normal LG. F = type FF motor units, R = type FR, I = type FI, S = type S. See text for explanation.

weight (-0.37, $p<0.0002$). These relationships are similar to those seen in MG (CHAPTER III).

Lateral gastrocnemius muscle fiber areas and innervation ratios. Muscle fiber areas for types FG, FOG, and SO fibers within each innervation compartment of LG are seen in Table 5-4. The overall values for each muscle fiber type in Table 5-7, were calculated based upon the proportions of motoneurons innervating each compartment (Weeks and English, 1985; see above). The overall means are similar to those in MG. Overall, and within each compartment, type FG fibers were significantly larger than types FOG and SO, which did not differ from one another.

Relative innervation ratios were calculated as % muscle fiber type divided by % motor unit type (Dum et al. 1982). The complicated muscle fiber architecture and compartmented arrangement of LG (English and Letbetter, 1982a,b) make analysis of absolute innervation ratios or specific tensions unreliable. In general, relative innervation ratios for LG motor units were similar to those for MG motor units.

Lateral gastrocnemius motoneuron electrical properties. Mean values for LG motoneuron electrical properties are also similar to those of MG motoneurons (Table 5-5). There were no differences ($p<0.01$) for any means between these muscles. The frequency distributions for motoneuron parameters were also similar to MG (Fig. 5-2).

Motoneurons segregate by motor unit type with respect to rheobase and input resistance (Fig 5-3A). Motoneuron type was defined by the ratio rheobase/input resistance (FF>18; 7<FR<18; S<7) and AHP half-decay time (FF+FI+FR: AHP <30ms; S: AHP>30ms; Zengel et al., 1985; CHAPTER III). These predictions were found to agree with motor unit type

Table 5-3. Muscle Unit Contractile Properties: Normal MG, LG, and Soleus.[a]

	FF	FI	FR	S	ALL
TWITCH TIME-TO-PEAK [b] (ms)					
MG [b]	29±1 (56)	28±2 (7)	26±1 (30)	61±4 (22)	34±2 (115)
LG	27±1 (66)	26±2 (4)	27±1 (17)	51±3 (15)	30±1 (102)
SOLEUS				82±4 (43)	82±4 (43)
TWITCH AMPLITUDE [b] (g-wt.)					
MG	18±1 (56)	4±1 (7)	1±0 (28)	0.2±0 (22)	9±1 (113)
LG	16±1 (66)	5±2 (4)	1±1 (17)	0.7±0 (15)	11±1 (102)
SOLEUS				2.0±0 (43)	2±0 (43)
TWITCH HALF-RELAXATION TIME [b] (ms)					
MG	24±1 (56)	27±2 (7)	26±1 (29)	74±8 (19)	33±2 (111)
LG	23±1 (65)	27±3 (4)	26±2 (15)	56±6 (15)	28±2 (99)
SOLEUS				103±5 (43)	103±5 (43)
MAXIMUM TETANUS (g-wt.)					
MG	61±3 (84)	20±3 (7)	13±1 (45)	7±1 (39)	35±2 (175)
LG	48±3 (66)	17±7 (5)	8±2 (17)	4±1 (15)	34±3 (103)
SOLEUS				8±1 (43)	8±1 (43)
FATIGUE INDEX					
MG	0±0 (82)	0.5±0 (7)	1.0±0 (45)	1.1±0 (40)	0.6±0 (174)
LG	0±0 (60)	0.5±0 (5)	1.0±0 (9)	1.1±0 (15)	0.4±0 (89)
SOLEUS				1.0±0 (40)	1.0±0 (40)
TWITCH/TETANUS [c]					
MG	.15±0 (56)	.08±0 (7)	.05±0 (28)	.07±0 (16)	.10±0 (107)
LG	.17±0 (63)	.10±0 (4)	.07±0 (9)	.05±0 (7)	.15±0 (83)
SOLEUS				.21±0 (38)	.21±0 (38)

a. Means ± SE (number of units).
b. Potentiated twitch.
c. Unpotentiated twitch/maximum tetanus.

determined by muscle unit contractile properties in 84% of sampled LG units. This compares favorably to results obtained in normal MG (86%: CHAPTER III; 95%: Zengel et al., 1985). Thus motoneuron type is an excellent predictor of motor unit type in cat LG, as well as in MG. Overall, log rheobase decreases as input resistance increases ($r = -0.69$, $p<0.0001$), as in MG (Fleshman et al. 1981;, Zengel et al. 1985; CHAPTER III). This relationship was not significant within any motor unit type.

Cat LG motoneurons segregate into fast and slow groups with respect to the AHP half-decay time: axonal conduction velocity relationship (Fig. 5-4A). With respect to these properties, we found 73% (11/15) of LG type S motoneurons within the normal MG range for type S units, and 88% (73/83) of LG fast (FF+FI+FR) motoneurons were within the MG fast range. AHP half-decay time alone distinguished between fast and slow motoneurons 92% of the time (92/100; AHP <30ms = fast, AHP > 30ms = slow). Across all units, AHP half-decay time and axonal conduction velocity were negatively correlated ($r = -0.42$, $p< 0.0001$), as in MG. This relationship was not significant within any motor unit type.

On the whole, cat LG motoneuron electrical properties and their interrelationships are as in cat MG motoneurons.

Relationships between LG motoneurons and muscle units. Motoneuron type, determined by membrane electrical properties, was an excellent predictor of motor unit type in LG as in MG (see above). This suggests a tight relationship between motoneuron properties in combination, and muscle unit speed- and fatigue-related properties. As in MG, relationships

Table 5-4. Muscle Fiber Areas By Innervation Compartment: Normal LG. [a,c]

	FG	FOG	SO	n/N [b]
LG_m	2979	1308	1356	373/3
LG_1	3363	1495	1294	448/3
LG_2	4157	2614	2552	300/2
LG_3	3673	1696	1956	448/3

a) As defined by English and Letbetter (1982a,b).
b) Number of muscle fibers/number of animals.
c) Values in um^2

Table 5-5. Motoneuron Electrical Properties: Normal MG, LG and Soleus. [a,b,c]

	FF	FI	FR	S	ALL
RHEOBASE (nA)					
MG	20±1 (70)	16±1 (6)	11±1 (37)	5±0 (34)	14±1 (147)
LG	22±1 (64)	16±1 (5)	8±1 (18)	5±1 (15)	17±1 (102)
SOLEUS				5±0 (38)	5±0 (38)**++
INPUT RESISTANCE (Mohms)					
MG	0.6±0 (56)	0.9±0 (3)	1.1±1 (30)	1.5±0 (28)	1.0±0 (117)
LG	0.6±0 (60)	0.8±0 (5)	1.3±0 (17)	2.0±0 (15)	1.0±0 (97)
SOLEUS				2.1±0 (36)**	2.1±0 (36)**++
RHEOBASE/INPUT RESISTANCE (nA/Mohms)					
MG	35±2 (55)	23±5 (3)	12±1 (30)	4±1 (28)	21±2 (116)
LG	36±4 (60)	22±2 (5)	8±1 (17)	3±1 (15)	25±3 (97)
SOLEUS				2±0 (36)*	2±0 (36)**++
AHP HALF-DECAY TIME (ms)					
MG	22±1 (54)+	19±2 (5)+	25±1 (31)	49±3 (28)	28±1 (118)+
LG	19±1 (63)*	22±2 (5)*	24±1 (18)	38±3 (15)	23±1 (101)*
SOLEUS				58±2 (38)**++	58±2 (38)**++
AXONAL CONDUCTION VELOCITY (ms)					
MG	97±1 (55)	100±4 (7)	99±2 (25)	81±2 (24)	94±1 (111)
LG	98±1 (62)	97±6 (5)	97±1 (17)	87±2 (15)	96±1 (99)
SOLEUS				74±1 (37)*	74±1 (37)**++

a. Means ± SE (number of units).
b. * = Significant difference from MG ($p<0.05$); ** ($p<0.01$).
c. + = Significant difference from LG ($p<0.05$); ++ ($p<0.01$).

Table 5-6. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types For Normal LG.

		P
Axonal Conduction Velocity	F>S	0.01
	(F,R)>S	0.05
Rheobase	F>(R,S)	0.01
Input Resistance	F<R<S	0.01
Rheobase/Input Resistance	F>(R,S)	0.01
AHP Half-Decay Time	(F,R)<S	0.01
Twitch Amplitude [a,b]	F>R	0.01
	F>(R,S)	0.05
Twitch Time-To-Peak [b]	(F,R)<S	0.01
Twitch HRT [b,c]	(F,R)<S	0.01
Tetanic Tension [a]	F>(R,S)	0.01
Fatigue Index	F<R<S	0.01
Twitch/Tetanus [d]	NS	0.01

a) The same result was obtained with raw data and data normalized by body weight.
b) Potentiated twitch.
c) HRT = half-relaxation time.
d) Twitch/tetanus = unpotentiated twitch/maximum tetanus.

between individual motoneuron properties and individual contractile properties are less clear.

In normal MG, the strongest relationship between a motoneuron property and a muscle unit property was between AHP half-decay time and twitch time-to-peak (Zengel et al., 1985; CHAPTER III). In LG the correlation between these variables was 0.55 ($p<0.0001$). This relationship did not hold within any motor unit type. The correlation between axonal conduction velocity and twitch time-to-peak was of similar magnitude, but inverse in sign ($r = -0.55$, $p<0.0001$). In LG, this relationship was significant for type FF units (-0.57, $p<0.0001$) and type FR units (-0.57, $p< 0.02$).

In general, the relationships between motoneuron and muscle properties in cat LG were very similar to those in MG, underscoring the similarity between the two gastrocnemius muscles.

SOLEUS

All motor units sampled in soleus were of type S (Table 5-2). All units had twitch time-to-peak values of >40ms and did not exhibit sag in an unfused tetanus (interstimulus interval = 1.25X time-to-peak; Burke et al., 1973; Mosher et al., 197-; McPhedran et al., 1965) Bagust (1974) and Dum et al. (1985b) observed a small number of fast-contracting units in cat soleus.

Soleus was nearly 100% type SO muscle fibers, although a small number of FOG fibers were found in three-of-eight muscles examined (137,155, and 234 FOG fibers, respectively). Based upon an estimate of 22,000-30,000 muscle fibers in soleus (Clark, 1931), FOG muscle fibers

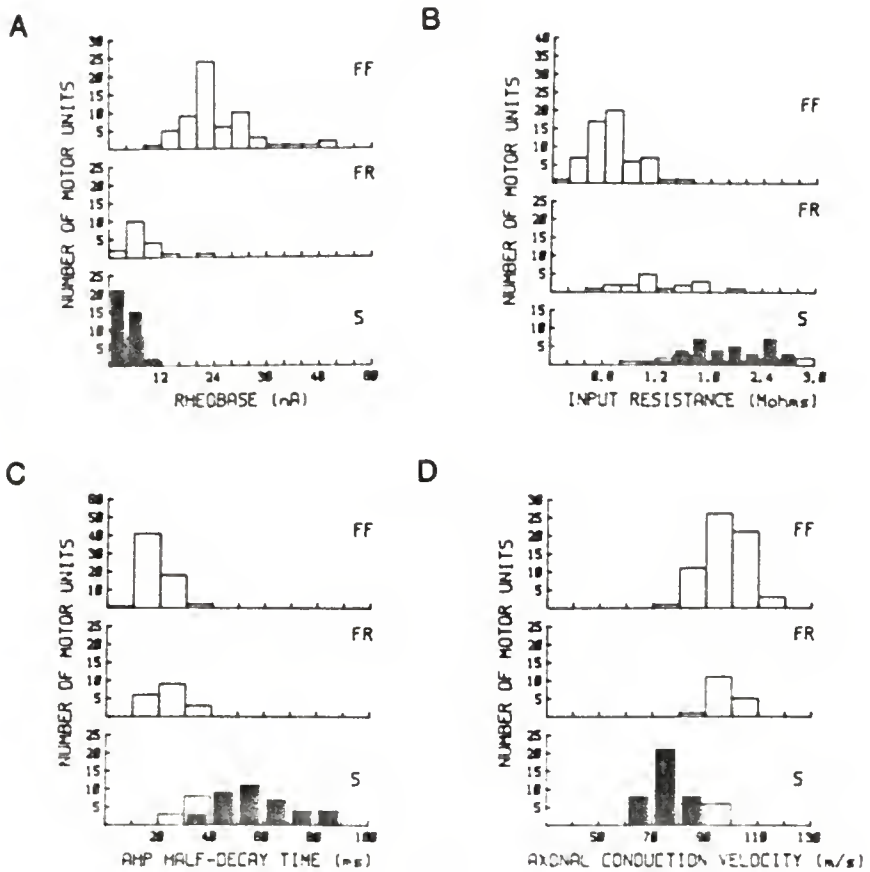

Figure 5-3. Frequency histograms for normal LG (unfilled) and soleus motoneuron properties. (A) Rheobase (B) Input resistance (C) Ahp half-decay time (D) axonal conduction velocity. See text for explanation.

comprise less than 1% of soleus fibers (Ariano et al. 1973; Burke et al. 1974; Henneman and Olson, 1965;).

Soleus muscle unit contractile properties. Overall means for all motor units combined were different in soleus than LG and MG, for all parameters measured. Observed values for soleus were similar to those of previous studies (Bagust, 1974; Burke et al. 1974; Mosher et al. 1972; McPhedran et al. 1965; Cope et al. 1983; Hammarberg and Kellerth, 1974). The differences in overall means reflect the different motor unit type distributions in these muscles, as well as differences in muscle fiber length and architecture (Sacks and Roy, 1982). Soleus S units differed from S units in LG and MG in having significantly longer twitch time-to-peak and twitch half-relaxation times ($p<0.01$; Burke et al. 1974; Hammarberg and Kellerth, 1974; Pinter et al. 1983). Fatigue resistance was similar for soleus, MG, and LG type S units (Hammarberg and Kellerth reported a slightly lower fatigue index in soleus units, $p<0.05$). Maximum tetanic force differed when expressed as a fraction of body weight, with soleus units generating more tension than gastrocnemius type S units (Burke et al. 1974). The distributions of motor unit contractile properties in soleus overlapped those of type S units in LG (Fig. 5-1) and MG (CHAPTER III).

Bagust and coworkers (1974a) reported that in some soleus units, twitch tension was depressed following tetanic activation. This extended an observation made earlier for whole soleus muscle (Brown and Von Euler, 1938). Burke (1980) reported that 13/20 cat soleus S units generated decreased twitch tension following tetanic stimulation, while all type S units in cat MG exhibited potentiation of the twitch

following tetanic stimulation. We found that 27/44 soleus units exhibited decreased twitch tension following a tetanus, as compared to 3/7 in LG type S units, and 5/20 in MG type S units.

In soleus, there was no significant relationship between twitch time-to-peak and twitch or tetanic tension for the overall sample or any motor unit type.

Soleus muscle fiber areas and innervation ratios. Soleus muscle fibers were almost exclusively type SO (Henneman and Olson, 1965; Burke et al. 1974; Fig. 5-7). Soleus type SO muscle fibers were larger in cross-sectional area than type SO fibers in the gastrocnemii (Table 5-7; Henneman and Olson, 1965; Burke, 1981; Gardiner et al. 1978). For the small sample of type FOG fibers from three animals (see above), we saw no difference in cross-sectional area between fibers of types FOG or SO. Soleus type FOG fibers were larger than type FOG fibers in MG or LG.

We assumed there were 145 motoneurons in the soleus pool (Boyd and Davey, 1968; Burke et al. 1977; Weeks and English, 1985). If one assumes 22,000-30,000 muscle fibers in cat soleus (Clark, 1931), this results in an average innervation ratio of 152-207 fibers (Burke et al. 1974).

Soleus motoneuron electrical properties. Mean values for soleus motoneuron electrical properties are found in Table 5-5. Mean soleus AHP half-decay time was significantly longer than for type S units of LG or MG ($p<0.01$; Hammarberg and Kellerth, 1974; Eccles et al., 1958; Kuno, 1959; Burke, 1981; Pinter et al. 1983). Input resistance was significantly higher in soleus motoneurons than in MG type S motoneurons ($p<0.01$), but not than in LG type S motoneurons (Burke et al. 1982; Burke, 1967; Burke, 1981; Pinter et al. 1983; Zengel et al. 1983).

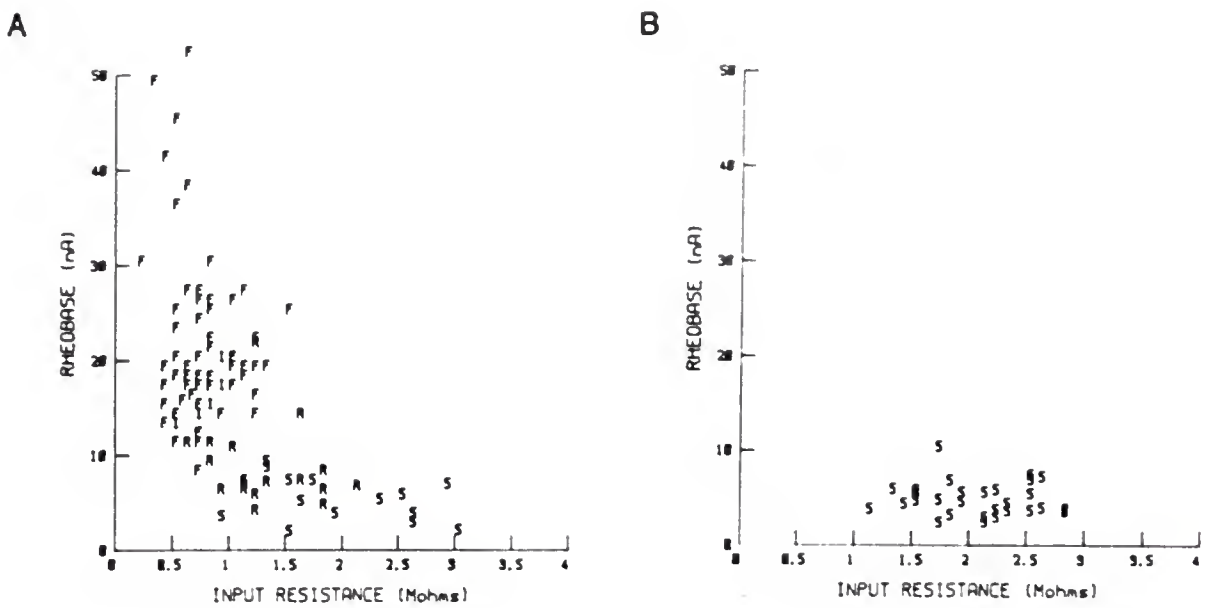

Figure 5-4. Relationships between rheobase and input resistance in normal LG and soleus motoneurons. F= FF units, I=FI, R=FR, S=S units. (A) LG. Note segregation by motor unit type. (B) Soleus. All motor units are type S and occupy the area on the graph occupied by type S motoneurons in LG.

Axonal conduction velocity was lower in soleus motoneurons than in LG type S units (0.01), but not from MG (Burke et al. 1982; Burke, 1981; Burke et al. 1974; see also Mosher et al. 1972; Bagust, 1974; Kuno, 1959). Mean rheobase did not differ from values for gastrocnemius type S motoneurons. Previous studies have reported that mean input resistance of soleus motoneurons was higher than mean values for motoneurons of 'fast' muscles (Eccles et al. 1958; Kuno, 1959; Burke et al. 1974; Cope et al. 1983). The ratio rheobase: input resistance tended to be lower in soleus motoneurons than in MG type S motoneurons. All of these differences tend to accentuate the properties characteristic of type S units in mixed muscles, such as MG or LG.

Frequency distributions for membrane electrical properties in soleus motoneurons overlap those for LG and MG type S units (Fig. 5-1; Hammarberg and Kellerth, 1974). AHP half-decay times for all soleus motoneurons were greater than 30ms, with many values longer than those seen in gastrocnemius type S motoneurons, (for AHP duration: Burke, 1967, 1981; Cope et al., 1983; Hammarberg and Kellerth, 1974).

The relationship rheobase:input resistance in soleus (Fig. 5-3) motoneurons was typical of type S units in MG (Zengel et al., 1985; CHAPTER III). This ratio alone categorized all soleus motoneurons as type S, in agreement with contractile-determined motor unit type. Rheobase (or log rheobase) and input resistance were not significantly correlated in the overall sample. This may be due to the use of data pooled from many experiments (Bagust, 1974).

Soleus motoneurons also were homogeneous with respect to the ratio AHP half-decay time/axonal conduction velocity (Fig. 5-3B;

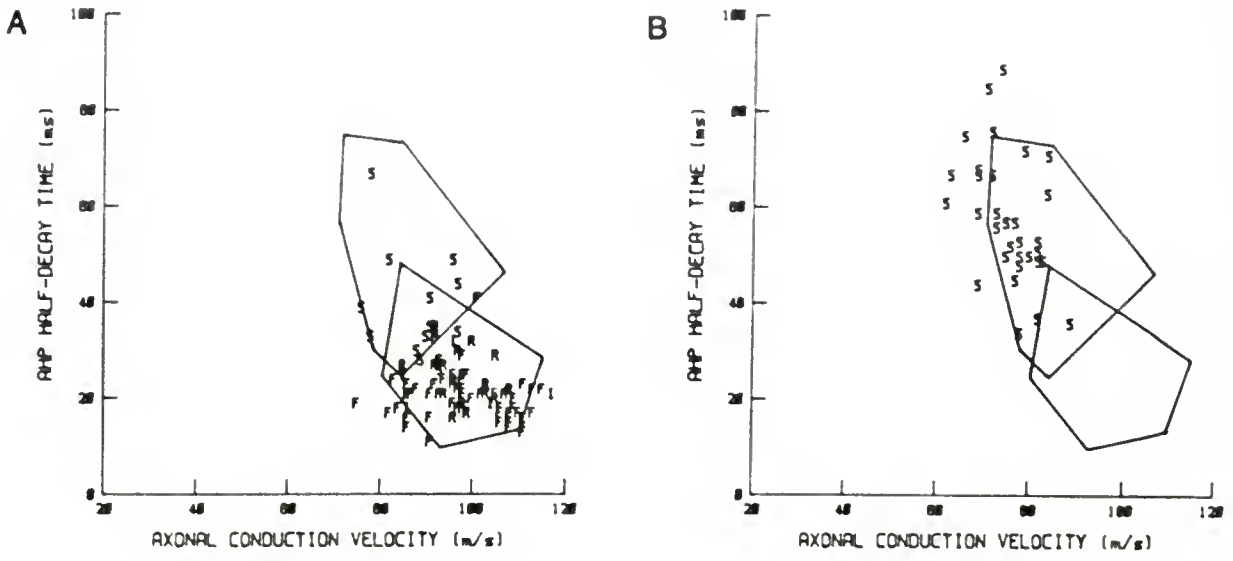

Figure 5-5. Relationship between AHP half-decay time and axonal conduction velocity in normal LG and soleus motoneurons. Symbols as in Fig. 5-4. (A) LG. Note overlapping but largely separate distributions for slow (S) and fast (F=FF, R=FR, I=FI) motoneurons. Solid lines outline the distribution of slow (upper) and fast motoneurons in MG (CHAPTER III). (B) Soleus. Note overlap with MG type S motoneurons (upper outlined area).

Kuno et al. 1974a,b). Most (68%) soleus motoneurons fell within the MG slow range for this relationship, those outside the MG range differed in having lower axonal conduction velocity and longer AHP duration. This supports the impression of soleus units being even "slower" than MG or LG type S units. These two variables are negatively correlated in soleus motoneuons ($r = -0.46$, $p<0.005$; Eccles et al. 1958; Kuno, 1959).

Relationships between soleus motoneuron electrical properties and muscle unit contractile properties. As mentioned above, AHP half-decay time and rheobase/input resistance each independently predicted motor unit type with 100% accuracy in soleus. This suggests a particularly tight correspondence between motoneuron type and motor unit type in this muscle.

As in LG, AHP half-decay time was correlated with twitch time-to-peak ($r = -0.46$, $p<0.005$). This was the strongest relationship between motoneuron and muscle unit properties in MG (CHAPTER III; Zengel et al. 1985). Huiszar et al. (1977) found this to be the strongest relationship in soleus as well. In the present study, the correlation between axonal conduction velocity and twitch time-to-peak was of similar strength ($r = -0.45$, $p< 0.009$; McPhedran et al. 1965; Bagust, 1974; Jami and Petit, 1975; but see Mosher et al. 1972). There were no significant correlations between axonal conduction velocity and twitch or tetanic tension (Burke, 1967; Mosher et al. 1972). This is likely due, at least in part, to use of pooled data of small samples from several animals (McPhedran et al. 1965; Bagust 1974; Jami and Petit, 1975). The largest sample from any single animal was six soleus units.

Table 5-7. Motor Unit Types, Muscle Fiber Types and Innervation Ratios for Normal MG, LG and Soleus.

MUSCLE FIBER TYPES

Histochemical Composition (%)

	FG	FOG	SO	n/N [a]
MG	57	28	15	19049/4
LG	65	16	18	15135/4
Soleus			100	x/8 [e]

Observed Mean Fiber Area (um^2)

				ALL
MG	3873	2264	1972	3104
LG	3695	1923	1961	3062
Soleus			3974	3974

EQUIVALENT MOTOR UNIT TYPE

% Of Population

	FF+FI	FR	S	n/N [a]
MG	48+4	24	24	176/16
LG	63+5	18	15	102/13
Soleus	0	0	100	41/14

Est. Number in Pool [d]

				ALL [b]
MG	134+11	67	67	280
LG	183+5	52	44	290
Soleus			145	145

CALCULATED VALUES

Relative IR [c]	FF+FI	FR	S
MG	1.1	1.2	0.6
LG	1.0	0.9	1.2
Soleus			1.0

a. Number of Cells/Number of Animals.
b. MG (Burke et al. 1977); LG,Sol (Weeks and English, 198-); MG, LG, and soleus (Boyd and Davey, 1968).
c. Relative Innervation Ratio = % Muscle Fiber Type/% Motor Unit Type.
d. Estimated as (All)(% motor unit type).
e. Counted number of type FOG fibers in mid-length cross-section. See Text.

Thus in soleus, as in MG and LG, there is a tight relationship between many motoneuron and muscle unit properties. The homogeneous motor unit type composition of soleus is accompanied by a relatively homogeneous motoneuron pool.

Discussion

This study examined the distribution of motor unit types, and properties of motoneurons and muscle units types in cat LG and soleus, and compared them to those of MG.

In general the distributions of LG motor unit types is similar to that of MG, exceptions being a higher proportion of fatiguable (FF) units, and fewer type S units in LG compared to MG. The distribution of muscle fiber types in LG was consistent with the distribution of motor unit types, including more type FG muscle fibers than in MG. We have confirmed that muscle fiber types are distributed in different proportions in the four compartments of LG (English and Letbetter, 1982b), and for a limited sample of motor units this difference seems to be consistent with localization of individual motor unit types.

Motoneuron electrical properties (rheobase, input resistance, AHP half-decay time, and axonal conduction velocity), as well as muscle unit contractile properties (twitch time-to-peak, twitch amplitude, tetanic tension, and fatigue index) of LG motor units were very similar to those of MG motor units of the same type. Relationships between and among motoneuron electrical properties and muscle unit contractile properties were also similar to MG. Motoneuron electrical type (Zengel et al. 1985) was an excellent predictor of motor unit type for LG units, as in MG.

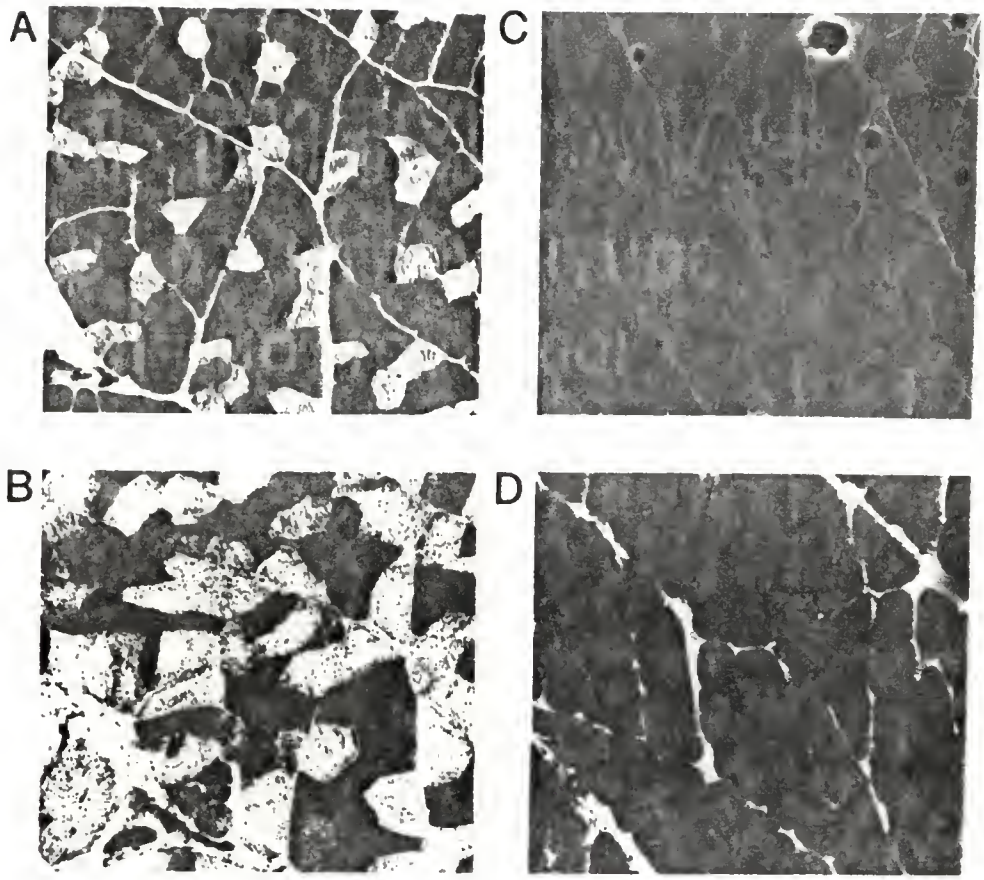

Figure 5-6. Photomicrographs of normal LG and soleus muscle histochemistry. (A) LG, myosin ATPase (pH 10.3) (B) LG, NADH-D (C) Soleus, myosin ATPase, pH 10.3 (D) Soleus, NADH-D.

All soleus motor units sampled were of type S, consistent with earlier reports (Mosher et al. 1972; Burke et al. 1973; Cope et al. 1983; Hammarberg and Kellerth, 1974). We saw a small, variable number of type FOG muscle fibers in soleus, suggesting the presence of a few fast-twitch motor units (McPhedran et al. 1965; Bagust, 1974; Burke, 1981; Dum et al. 1985b).

Soleus units differed from type S units in LG and MG, with respect to twitch time-to-peak and twitch half-relaxation times (Burke et al. 1974; Hammarberg and Kellerth, 1974), and maximum tetanic force (normalized by body weight; Burke et al. 1974). A large percentage of soleus units exhibited depressed twitch response following tetanic stimulation, unlike MG or LG type S units (Burke et al. 1974; Burke, 1980). Soleus units were similar to gastrocnemius type S units in resistance to fatigue. Soleus motor units had lower mean innervation ratio and larger muscle fibers than gastrocnemius type S units. Collectively these data suggest that soleus muscle units and gastrocnemius type S muscle units are different populations (Burke, 1981).

Soleus units also differed from type S units of LG and MG with respect to motoneuron electrical properties. Input resistance was higher while axonal conduction velocity (Burke et al. 1974) and the ratio rheobase:input resistance were lower in soleus motoneurons than MG or LG type S motoneurons. Mean AHP half-decay time was longer in soleus motoneurons (for AHP durations: Eccles et al. 1958; Kuno, 1959; Hammarberg and Kellerth, 1974; Burke, 1981; Cope et al. 1983). All of these differences in motoneuron and muscle unit properties accentuate

the characteristics of type S motor units and suggest that soleus motoneurons are different from gastrocnemius type S motoneurons. Mean rheobase was similar in gastrocnemius type S motoneurons and soleus motoneurons (Cope et al. 1983).

All soleus motoneurons were categorized as type S on the basis of AHP half-decay time or the ratio rheobase:input resistance (alone or in combination; Zengel et al. 1985) Motoneuron type was 100% accurate in estimating motor unit type in soleus units. Thus there seems to be a particularly strong relationship between motoneuron electrical properties (in combination) and muscle unit properties (as reflected in motor unit type) in soleus.

Functional and Developmental Considerations

The similar composition of motor unit types between MG and LG is consistent with similar phasic activation patterns of the two muscles during locomotion (Rasmussen et al. 1978; Smith et al. 1977), while soleus is more tonically active (Rasmussen et al. 1978; Smith et al. 1977). The higher proportion of fatiguable units in LG compared to MG might indicate that an even greater proportion of the LG muscle is activated only during maximal contractions (Hammant, 1977). It is unclear why ankle extension requires four muscles with similar mechanical action (the triceps surae complex and plantaris). Perhaps architectural differences (Spector et al. 1980; Sacks and Roy, 1982) may allow different muscles (Spector et al. 1980), or compartments (English, 1984; Russel et al. 1982) to provide force, velocity, or elastic energy storage at slightly different parts of the step cycle, or in different tasks.

Ontogenetically (Bardeen, 1906) and phylogenetically (Bardeen, 1906; McMurrich, 1906), soleus, plantaris and the two gastrocnemius muscles arise from a common primordium. The initial split is between plantaris and the triceps surae, followed by MG vs. LG-soleus. The last division is between LG and soleus. In the oppossum (Didelphis virginiana; Peters et al. 1984) and certain australian marsupials (Lewis, 1962), the LG and soleus muscles are fused, soleus forming a relatively distinct deep (to the tibia) and distal compartment of a combined LG-soleus muscle (histochemically uniform; Peters et al. 1984). In the oppossum, soleus is innervated by a separate branch of the LG-soleus nerve (Peters, 1984), as in the cat (English and Letbetter, 1982a). Oppossum fast-twitch LG-soleus units were found to be slower and generate more tension than MG units (Peters et al. 1984). All oppossum triceps surae motor units (fast and slow) were highly fatigue resistant (Peters et al. 1984).

In the cat, we found LG fast-twitch units to be similar in size and speed to those in MG. The primary difference was the presence of more fatiguable (FF) units in cat LG compared to MG. It appears that in addition to anatomical separation of soleus and LG muscle in the cat (vs. oppossum), the individual muscles are specialized with respect to motor unit type composition: soleus being essentailly 100% slow and fatigue-resistant, and LG primarily fast and fatiguable. Perhaps the greater proportion of fatiguable units in LG vs. MG reflects, in part, the developmental and evolutionary division of the common LG-soleus muscle into two specialized portions. The present work indicates that motoneuron electrical properties and muscle unit properties are strongly

related in all three triceps surae muscles. Neuronal regulation of muscle unit properties (e.g. Buller et al. 1960; Burke, 1980; Gordon and Stein, 1982a; Chan et al. 1982; CHAPTER III) is a possible mechanism in which specialization of muscle units is preceded by specialization within the motoneuron pool. Alternatively, specialization could occur by modification of the internal developmental program of the muscle fiber, with motoneuron properties influenced by a feedback mechanism (CHAPTER VII).

CHAPTER VI
CROSS-REINNERVATION OF LG AND SOLEUS MUSCLES BY MG MOTOR NERVE: SELECTIVITY OF REINNERVATION AND MOTONEURON INFLUENCE UPON MUSCLE

Introduction

The mammalian neuromuscular system has been an important model for investigating interactions between cell types. The classic work of Buller *et al.* (1960) utilized the technique of surgically re-routing a foreign, heterogeneous nerve (flexor hallucis longus, FHL) into the homogeneous, slow soleus muscle of the cat, and *vice versa* (X-reinnervation). This study model revealed that the identity of the innervating nerve strongly influenced the phenotypic expression of the muscle. An important observation was that while the twitch speed of the formerly fast FHL was completely converted to the normal, slow soleus condition, the reverse was not true. Soleus muscle twitch speed was incompletely converted to that of normal FHL.

The importance of neural identity in determination of muscle properties, and the incomplete conversion of slow muscle by a mixed nerve, has since been demonstrated several times for whole muscle contractile (Prewitt and Salafsky, 1967; Eccles *et al.* 1962; Buller and Lewis, 1965; Close, 1965; Close and Hoh, 1969; Young *et al.* 1984; Luff, 1975; Luff *et al.* 1984), and biochemical properties (Buller *et al.* 1969; Mommaerts *et al.* 1977). Other studies have examined muscle histochemistry (Romanul and Van der Meulen, 1967; Dubowitz 1967; Edgerton *et al.* 1980; Chan *et al.* 1982; Lewis *et al.* 1982;

Burke et al. 1979; Dum et al. 1979, 1985a,b; Burke, 1980) or single motor unit contractile properties (Dum et al. 1979; Burke et al. 1979; Burke, 1980; Bagust et al. 1981; Chan et al. 1982; Lewis et al. 1982; Dum et al. 1985a,b). Only Lewis et al. (1982) and Burke and coworkers (Burke, 1980; Burke et al. 1979; Dum et al. 1979; Dum et al. 1985a,b) analyzed the results in terms of motor unit types.

Incomplete conversion of soleus by mixed nerves could reflect unwanted self-reinnervation, different muscle mechanics and architecture, or differential response of motor unit types to nerve section and reinnervation (Dum et al. 1985a).

Experiments in which mixed muscles were chronically stimulated suggest that the amount and/or frequency of muscle activity plays an important role in the transformation of muscle fiber properties (reviewed in Salmons and Henrickson, 1981; Pette, 1984).

This study utilized the cross-reinnervation model to test whether reinnervation of foreign muscles was selective with respect to motor unit types. The MG nerve was surgically re-routed into the combined lateral gastrocnemius-soleus (LG-S) nerve (Fig. 2-1). This gave MG motoneurons a 'choice' between innervating LG, a mixed muscle with muscle fiber type composition similar to MG (CHAPTER V; English and Letbetter, 1982b; Ariano et al. 1973), and soleus, which contains nearly 100% slow fibers (Ariano et al. 1973; Burke, 1981; Burke et al. 1974; Chan et al. 1982; Edgerton et al. 1980; CHAPTER V). These three muscles form a functionally synergistic group, the triceps surae. We tested whether reinnervation of LG and soleus is selective according to motor unit type, examined the degree to which whole muscle and muscle unit

contractile properties were determined by the innervating motoneuron, and tested whether the particular muscle innervated influences the expression of motoneuron electrical properties. The influence of connection to particular muscles on expression of motoneuron electrical properties is addressed in CHAPTER VII.

Results

Whole Muscle

Values for whole muscle wet weights and contractile properties are found in Table 6-1. There were no significant differences between nine month cross-reinnervated LG (longX-LG) and control LG. LongX-soleus differed from control soleus in all parameters. Values for speed-related parameters (time-to-peak, half-relaxation time, half-rise time) of longX-soleus were 'faster', although not as 'fast' as control MG or LG (Buller *et al.* 1960; Close, 1965; Close and Hoh, 1969; Luff, 1975; Prewitt and Salafsky, 1967; Burke, 1980; Dum *et al.* 1979; Chan *et al.* 1982; Bagust *et al.* 1981; Lewis *et al.* 1982). Wet weight and twitch tension were reduced from control soleus (75% and 59%, respectively). The longX-soleus muscles were red in gross appearance, as in normal soleus.

After ten weeks cross-reinnervation of LG (MedX-LG) speed-related properties were similar to normal LG, but twitch tension and muscle weight had not recovered to longX levels. MedX-soleus tensions and muscle weights were also incompletely recovered. Twitch half-rise time and half-relaxation time of medX-soleus were incompletely altered relative to longX-soleus.

Table 6-1. Whole Muscle Properties: Cross-reinnervation.[a,c]

	LONG		TEN WEEKS	
	X-LG	X-SOLEUS	X-LG	X-SOLEUS
Twitch Time-To-Peak[b] (ms)	30±1	49±5**	32±1	51±11
Twitch Half-Rise Time[b] (ms)	10±1	13±1**	12±1	18±4
Twitch Half-Relaxation Time[b](ms)	18±1	72±7**	26±1	108±25
Twitch Tension[b] (g-wt.)	1653±148	407±51**	904±101**	195±34**
Muscle Weight (g)	7.2±1	2.5±0**	5.1±0	1.6±0*
Muscle Wt./Cat Wt. (g/kg)	2.5±0	0.9±0**	2.1±0	0.7±0*
Twitch Tension/Cat Weight (g-wt./kg)	577±56	141±13**	377±45*	79±9**

a. Means±SE (for long-X LG and SOLEUS, n= 15; for ten weeks n = 4).
b. Twitch at muscle length at which maximum tension was obtained.
c. * = significant difference from same muscle in unoperated normal animals ($p<0.05$); ** ($p<0.01$).

Table 6-2. Percent Motor Unit Types.

	FF	FI	FR	S	n/N[a]
Normal MG	48	4	24	24	176/16
Self-reinnervated MG	46	4	24	26	72/4
LongX (all)	35	7	25	33	138/10
X-LG	51	5	27	17	93/10
X-Soleus	2	11	20	67	45/10
MedX (all)	30	20	13	37	54/4
X-LG	40	28	15	18	40/4
X-Soleus	0	0	7	93	14/4
Normal Soleus				100	41/14
Normal LG	63	5	18	15	102/13

a. Number of cells/number of animals.

Reinnervation of muscle by the original nerve has been a problem in previous studies of cross-reinnervation (Buller et al. 1960; Cohen, 1978; Romanul and Van Der Meulen, 1967; Edgerton et al. 1980; Bagust et al. 1981; Chan et al. 1982; Burke, 1980). To assess the degree of unwanted self-reinnervation, we recorded soleus and LG muscle twitch tension in response to stimulation of the [illegible]-S nerve (proximal to nerve cuff), as well as in response to the MG nerve. The MG and LG muscles were not completely separated, in order to prevent compromising the blood supply. This mechanical linkage between the MG and LG muscles complicated the interpretation of the results. In unoperated control animals the ratio: tension after 'correct' nerve (e.g. for unoperated MG muscle, the MG nerve is 'correct'; for X-MG, the LG-soleus nerve is 'correct') stimulation: tension after 'incorrect' nerve (e.g. LG-soleus nerve for unoperated MG) stimulation, was 0.06 for LG and 0.03 for soleus (n = 8). In X-reinnervated LG the ratio was 0.17 (significant difference from control) and in longX-soleus was 0.02 (no significant difference, n= 8). For medX-LG and medX-soleus this ratio did not differ from the unoperated normal level.

A better test of the amount of unwanted self-reinnervation was performed on six longX-operated and five normal animals. In these animals, whole muscle twitches were remeasured following single unit studies. The LG and MG muscles were then separated 90-100%, and twitch tensions recorded again. In unoperated control animals, separation of the muscles resulted in no mechanical response measured in the LG tendon in response to stimulation of the 'incorrect' nerve. In longX-LG the ratio tension to stimulation of the 'correct' nerve: tension to

'incorrect'stimulation, ranged from 0-0.04. These six animals included the full range of ratios observed for the unseparated muscles (0.09-0.27). All crosses were thus at least 96% 'pure' (inverse of ratio). On this basis, we conclude that all X-reinnervations reported in this study were 96% 'pure', or better. The question of purity of cross-reinnervation is important for interpretation of whole muscle contractile responses and muscle histochemistry, but does not affect the single unit studies. The muscles used for histochemical analysis of LG were all from animals in which muscle separation was performed.

Motor Unit Type Distribution

The overall sample of longX-reinnervated motor units was not in normal MG proportions (Table 6-2), unlike self-reinnervated MG (CHAPTER III; see also Gordon and Stein, 1982a; Dum et al. 1979). There were fewer type FF units and more type S units in the longX-reinnervated population (Table 6-2). When the sample was divided into two groups: units in longX-LG and those in longX-soleus, the distribution of motor unit types in longX-LG was not different from control MG, but the longX-soleus distribution differed significantly. LongX-soleus contained a predominance (67%) of type S units, and only one type FF unit (Table 6-2). Fast motor unit types were found (33-75% of sample) in eight of nine animals in which soleus units were isolated. There are three possible interpretations for these results. First, the distribution might be explained by selective reinnervation of soleus muscle by type S motoneurons. Second, soleus might receive a random sample of MG

motoneurons, but 'resists' motor unit type conversion. Finally, the results may reflect sampling bias. We feel the latter explanation is unlikely as the sample size was large (138 motor units), and the soleus pattern was consistent between animals.

Normal LG is innervated by approximately 290 motoneurons (Boyd and Davey, 1968; Weeks and English, 1985). Normal soleus is innervated by approximately 140-150 motoneurons (Boyd and Davey, 1968; Burke et al., 1977; Weeks and English, 1985). On this basis one would predict that 67% of regenerating MG axons should enter endoneurial tubes leading to LG muscle, with the remaining one third innervating soleus. Approximately 10% (16/154) of sampled longX motoneurons failed to make functional reconnection with extrafusal muscle fibers, similar to self-reinnervation (Foehring et al. 1986a; Kuno et al. 1974b). Of the units which made functional connection with muscle, 67% were in longX-LG (82/123), and 33% in longX-soleus (41/123). This suggests that sorting of MG motoneurons into the LG or soleus nerves was by non-selective growth into endoneurial tubes leading to the two muscles. Since the longX-LG units represent two thirds of the motor units, and since longX-LG motor unit types are in proportions similar to normal MG, it is unlikely that the distribution of the remaining one-third of units (longX-soleus) could be explained by selective innervation.

We can address the question of selective reinnervation of soleus by calculating the number of type S motoneurons required to account for the total X-reinnervated distribution, and comparing this figure to the number of type S motoneurons available in the MG motoneuron pool. Unoperated control MG contains 280 motoneurons (Boyd and Davey, 1968;

Burke et al. 1977). Approximately 24% (67) of these are type S (CHAPTER III; Fleshman et al. 1981; Burke, 1981). In the present study, 10% of motoneurons did not make functional connections with either muscle, thus approximately 252 motoneurons innervated either longX-LG or longX-soleus. About 67% (169) of these motoneurons innervated longX-LG (33% = 83 in Soleus). If we assume that MG motoneurons converted LG muscle fibers, as in self-reinnervation of MG, then the longX-LG population included 29 type S motoneurons [17% of population (Table 6-2) times 169 motoneurons]. Of 83 soleus units, 56 would be type S (67% of population; Table 6-2). Thus 85 (29+56) type S motoneurons would be required to account for the overall motor unit type distribution. This is significantly more (Chi Square) than the 67 type S motoneurons present in controls (or 67-10% = 60 available, assuming random loss of all motoneuron types with reinnervation; see CHAPTER III). This is true even if one assumes that all of the 10% of motoneurons without functional connection to either muscle were originally fast (FF+FI+FR) motoneurons. (A similar argument and conclusion can be found in Dum et al. 1985b).

The above calculations argue against selective reinnervation by motor unit type as the explanation for the observed distribution in longX-LG and longX-soleus. This conclusion is consistent with previous reports suggesting that selective reinnervation does not occur in mammalian twitch muscle systems (reviewed in Grinnell and Herrera, 1981). We conclude that the most likely explanation is that a full complement of MG motoneuron types innervate soleus muscle, and that soleus muscle fibers 'resist' conversion by MG motoneurons. This is

consistent with previous observations of incomplete conversion of motor unit types when cat soleus was directly innervated by a mixed muscle nerve (Burke, 1980; Burke et al. 1979; Chan et al. 1982; Bagust et al. 1981). Lewis and colleagues (Lewis et al. 1982) felt that the incomplete conversion of soleus whole muscle properties by a mixed nerve (flexor digitorum longus: FDL) was not due to failure of individual muscle fibers to convert, but due to the small size of fast motor units in longX-soleus.

Ten weeks post-operatively, we found that 23% of motoneurons sampled did not elicit muscle tension (non-contracts; CHAPTER III). As in self-reinnervation of MG, this suggests that some motoneurons destined to make functional connections with muscle had not yet done so. Approximately 74% of motoneurons with functional connection to muscle innervated LG (40/54), and 26% innervated soleus (14/54). The slightly greater proportion in LG at this stage, relative to nine months, may reflect the greater distance for growth into soleus. In medX-LG there was a similar fast:slow motor unit distribution to longX-[illegible] (Table 6-2), but as in ten week self-reinnervated MG, there were more fatigue-resistant units at ten weeks, than at ten months.

MedX-soleus contained 93% (13/14) type S units, with one type FR unit. This could indicate that fast axons contact muscle fibers later than slow axons, or that a considerable amount of motor unit type conversion occurs after this time. Alternatively, the paucity of fast units may be an artifact of the small sample size. The low proportion of fast units is consistent with the slower whole muscle twitch at ten weeks than at nine months.

LongX-LG Motor Unit Properties

X-LG muscle unit contractile properties. Because of differences in muscle architecture and mechanics among MG, LG, and soleus (e.g. cross one joint or two; angle of pennation, series elasticity), comparison of muscle unit contractile properties was made between the same muscle (MG or LG or soleus) in control and reinnervated conditions (Dum et al. 1985a).

There were few differences between longX-LG and control LG muscle unit contractile properties (Table 6-3, Fig. 6-1). Overall mean twitch time-to-peak was slightly shorter (also for type FF units). The overall fatigue index was higher and twitch:tetanus ratio was lower in the longX-LG (also for type FF motor units). Type S units had increased mean tetanic tension in longX-LG compared to control LG (also true when normalized by body weight; similar to self-reinnervated MG type S units; CHAPTER III). Unlike self-reinnervated MG, the tetanic and twitch tensions of longX-LG type FF units were not reduced. The distributions for all muscle unit contractile properties in longX-LG were similar to normal LG, with slightly more variability (Fig. 6-1). The significance of differences between motor unit types for contractile properties are seen in Table 6-6.

After ten weeks recovery, X-LG muscle units had recovered speed-related properties to normal LG levels, but twitch and tetanic tensions were incompletely recovered. More medX-LG units were resistant to fatigue (Tables 6-2, 6-3, Fig. 6-7). This was similar to muscle unit properties observed after ten weeks self-reinnervation of MG (CHAPTER IV).

Table 6-3. Muscle Unit Contractile Properties: Normal vs. Cross-Reinnervated.[a,b]

	FF	FI	FR	S	ALL
TWITCH TIME-TO-PEAK [c] (ms)					
MEDX-LG	25±1 (16)	27±1 (11)	27±1 (6)	54±4 (7)	31±2 (40)*
LONGX-LG	23±1 (45)**	23±2 (5)	27±1 (24)	49±5 (15)	29±1 (89)**
CONTROL LG	27±1 (66)	26±2 (4)	27±1 (17)	51±3 (15)	30±1 (103)
MEDX-SOL			30 (1)	66±6 (12)	63±6 (13)
LONGX-SOL	21 (1)	26±1 (5)	31±2 (9)	68±3 (30)**	55±4 (45)**
CONTROL SOLEUS				82±4 (43)	82±4 (43)
TWITCH TENSION [c] (g-wt.)					
MEDX-LG	6±3 (15)**	2±1 (11)	0±0 (6)*	0±0 (7)*	3±1 (39)**
LONGX-LG	13±2 (45)	5±3 (5)	1±0 (24)	1±0 (15)	7±1 (89)*
CONTROL LG	16±1 (66)	5±2 (4)	1±1 (17)	1±0 (15)	11±1 (103)
MEDX-SOL			0 (1)	1±0 (7)*	1±0 (14)*
LONGX-SOL	1 (1)	1±0 (5)	1±1 (9)	2±0 (30)	2±0 (45)
CONTROL SOLEUS				2±0 (43)	2±0 (43)
TWITCH HALF-RELAXATION TIME [c] (ms)					
MEDX-LG	22±2 (15)	22±2 (10)	19±2 (4)	54±5 (6)	27±2 (35)
LONGX-LG	20±1 (42)	20±3 (5)	27±2 (19)	57±7 (12)	28±2 (78)
CONTROL LG	23±1 (65)	27±3 (5)	26±2 (15)	56±6 (15)	28±2 (99)
MEDX-SOL			38 (1)	63±8 (12)	61±7 (13)
LONGX-SOL	18 (1)	26±2 (5)	41±4 (8)	82±6 (26)**	65±6 (40)**
CONTROL SOLEUS				103±5 (43)	103±5 (43)
MAXIMUM TETANIC TENSION (g-wt.)					
MEDX-LG	32±7 (16)*	15±3 (11)	7±1 (6)	4±2 (7)	19±3 (40)*
LONGX-LG	51±5 (42)	31±4 (5)	13±3 (25)	8±1 (16)**	33±3 (93)
CONTROL LG	48±3 (66)	17±7 (5)	8±2 (17)	4±1 (15)	34±3 (103)
MEDX-SOL			17 (1)	7±2 (13)	8±2 (14)
LONGX-SOL	3 (1)	3±0 (5)	7±3 (9)	20±3 (30)**	15±2 (45)
CONTROL SOLEUS				8±1 (43)	8±1 (43)
FATIGUE INDEX					
MEDX-LG	.2±0 (14)**	.5±0 (11)	1±0 (5)	1.1±0 (6)	.5±0 (36)
LONGX-LG	.1±0 (41)**	.4±0 (5)	1±0 (23)	1.1±0 (13)	.5±0 (82)**
CONTROL LG	0±0 (60)	.5±0 (5)	1±0 (9)	1.1±0 (15)	.4±0 (89)
MEDX-SOL			0.8 (1)	0.9±0 (10)	0.9±0 (11)
LONGX-SOL	0.2 (1)	0.4±0 (5)	0.9±0 (9)	0.9±0 (27)	0.8±0 (42)**
CONTROL SOLEUS				1.0±0 (40)	1.0±0 (40)

a. Means ± SE (number of units).
b. * = Significant difference from same muscle in unoperated control sample ($p<0.05$); ** ($p<0.01$).
c. Potentiated twitch.

Overall means, and means by motor unit type for longX-LG contractile properties, were similar to those of control LG. We conclude that LG and MG muscle fibers respond to MG motoneurons in a similar manner. The time course of recovery of muscle unit contractile properties follows a similar pattern for self-reinnervation of MG and X-reinnervation of LG by MG motoneurons.

X-LG muscle histochemistry. Three longX-LG muscles were analyzed for distribution of muscle fiber types (Table 6-4). Values for the entire muscle were calculated by assuming that innervation ratios were similar for all compartments, and that the relative proportions of motoneurons innervating each compartment were as in unoperated control LG (Table 6-4; see Weeks and English, 1985). We found that longX-LG contained 64% type FG fibers, 13% FOG fibers, and 23% type SO fibers (Table 6-5). This is similar to self-reinnervated MG (CHAPTER III) and to normal LG (CHAPTER V). There is a slight trend towards increased type SO, and decreased type FOG fibers in longX-LG, as in self-reinnervated MG (CHAPTER III; see also Lewis et al. 1982).

An unexpected finding was that the LGm compartment of longX-LG muscles contained 88% fast (types FG+FOG, mostly type FG) muscle fibers, similar to controls (95% fast; CHAPTER V). This was consistent with a subjective impression that the medial head of LG was distinctly 'white' in appearance in all X-reinnervated LG muscles, as in normal LG (unpublished observations). The proportions of muscle fiber types in the other three compartments were very similar to each other, and nearly identical to the overall mean values for the entire muscle (Table 6-4). For each of the three animals, the proportions of muscle fiber types in

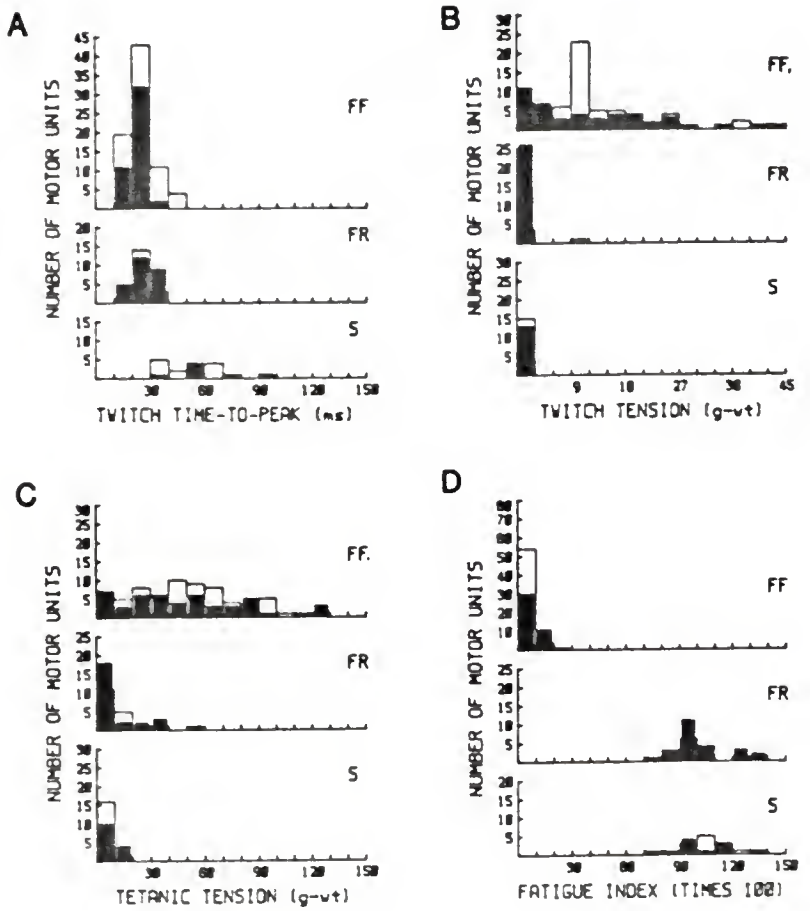

Figure 6-1. Frequency histograms for normal LG (unfilled) and longX-LG (filled) muscle unit contractile properties. (A) potentiated twitch time-to-peak ((B) potentiated twitch amplitude C) Tetanic tension (D) fatigue index. See text for explanation.

the LG1, LG2, and LG3 compartments varied randomly about the overall mean values for that muscle. Comparison of the same compartment between animals, indicates a random pattern of innervation for LG1, LG2 and LG3. The muscle fibers in the LGm compartment were predominately type FG in all three animals, with very few type SO fibers. The LGm compartment of LG may be similar to soleus in that it 'resisted' alteration of muscle fiber types by motoneurons, although the predominent fiber type in LGm is FG rather than SO.

X-LG muscle fiber areas and innervation ratios. We estimate that longX-LG is innervated by only 60% of its normal number of motoneurons (Table 6-8), yet whole muscle twitch tension was only reduced to 78% of normal LG. The reduced number of units is partially compensated for by increased mean muscle fiber area (Table 6-8), and increased average innervation ratio (Table 6-8).

Muscle fiber areas and relative innervation ratios are found in Table 6-8. Mean tetanic force was increased for type S motor units in longX-LG (Table 6-3). This was due largely to increased innervation ratio. Type FF units had tetanic tensions similar to normal LG. The decreased innervation ratio for type FR units, and increased innervation ratios of types FF and S units, suggest that type FR axons may be at a disadvantage in capturing muscle fibers during reinnervation of adult muscle (CHAPTERS III, IV).

MedX-LG muscle fiber areas were smaller than ten months post-operatively. The mean value medX-LG type FG fiber area was 1767um^2, for type FOG fibers 1169um^2, and for type SO fibers 1030um^2. The

Table 6-4. Percent Muscle Fiber Types By Innervation Compartment[a] (LONG-X).

		%FG	%FOG	%SO	n
FE35	LG_m	83	5	12	1009
	LG_1	56	8	36	960
	LG_2	67	6	27	996
	LG_3	65	19	16	961
	OVERALL [c]	66	11	23	
FE36	LG_m	85	6	15	902
	LG_1	74	14	12	804
	LG_2	61	31	8	675
	LG_3	56	0	44	662
	OVERALL [c]	64	14	22	
FE37	LG_m	76	11	13	673
	LG_1	62	19	19	699
	LG_2	59	5	36	669
	LG_3	63	17	20	839
	OVERALL [c]	63	13	24	
GRAND MEANS FOR 3 ANIMALS:					
	LG_m	81	7	13	
	LG_1	64	14	22	
	LG_2	62	14	24	
	LG_3	61	12	27	
	OVERALL [c]	64	13	23	

a) As defined by English and Letbetter (1982a,b).
b) Number of muscle fibers sampled.
c) Weighted average assuming 12% of motor units in LG^m, 19% in LG^1, 34% in LG^2, and 35% in LG^3. See text for details.

recovery of tension generally followed the increase of muscle fiber area from medX-LG to longX-LG.

X-Soleus Motor Unit Properties

X-Soleus muscle unit contractile properties. We found 33% fast (FF+FI+FR) motor units in longX-soleus. The primary criterion for separation into fast and slow motor units was time-to-peak of the potentiated twitch (<40ms = fast). Four of thirty type S motor units exhibited 'sag' (times-to-peak = 68,62,55, and 58 ms) and four of nine type FR units did not sag (times-to-peak = 28,31,30, and 28 ms). The proportions of fast motor units observed in X-reinnervated soleus differ greatly between laboratories. On the basis of 'sag' Chan et al. (1982) reported 39% fast units in cat soleus innervated by FHL motoneurons and Burke (1980) saw no fast units in soleus innervated by flexor digitorum longus (FDL). On the basis of twitch contraction time, Lewis et al. reported 64% fast units in soleus (innervated by FDL). The basis for the different results between labs is unclear, although variations in surgical technique, age of cats, and activity of animals may contribute.

The overall means for all contractile parameters except twitch and tetanic tension, were significantly different in long X-soleus than in normal soleus, reflecting the presence of a population of fast (FF+FI+FR) units not seen in normal soleus (Table 6-3, Fig. 6-2). The differences between motor unit types are shown in Table 6-6.

Twitch time-to-peak and half-relaxation time were shorter than in normal soleus (Chan et al. 1982; Burke et al. 1979; Bagust et al. 1981). Within the type S units, the distribution of times-to-peak was shifted

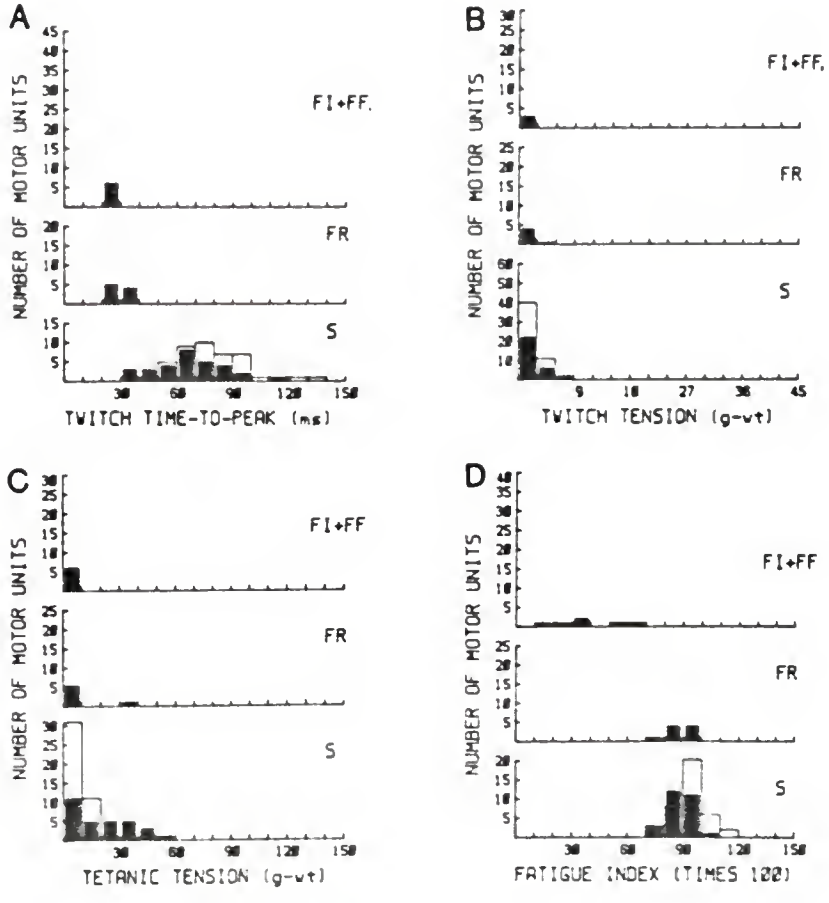

Figure 6-2. Frequency histograms for normal soleus (unfilled) and longX-soleus (filled) muscle unit contractile properties. (A) potentiated twitch time-to-peak (B) potentiated twitch amplitude (C) Tetanic tension (D) fatigue index. See text for explanation.

towards lower values, with a few units below the range of normal soleus (Fig. 6-2B). Overall fatigue index was reduced due to the presence of types FI and FF units (fatigue index of type S unchanged; Fig. 6-2D, Table 6-3). Most fast units in longX-soleus were fatigue-resistant (Table 6-2).

Overall, there were no significant differences for twitch or tetanic tension (Table 6-3). The single type FF unit sampled in longX-soleus was small (Table 6-3, Fig. 6-2A,C), and somewhat resistant to fatigue (fatigue index = 0.20), but otherwise typical of type FF units in unoperated MG or LG. Edgerton et al. (1980) reported a single fatiguable unit in soleus innervated by FHL nerve. Type FI units were also small but otherwise typical of MG or LG type FI units. Type FR units in longX-soleus were typical of type FR units in MG or LG except that half-relaxation time was longer. Thus, except for their small size (see also Bagust et al. 1981; Lewis et al. 1983), fast units in longX-soleus exhibit properties typical of fast units in the gastrocnemii. The size of fast units in long longX-soleus is consistent with 'resistance' to motoneuron-induced change in muscle unit properties of soleus muscle fibers and/or a disadvantage for fast motoneurons in capturing soleus muscle fibers.

Type S units in longX-soleus differed from normal soleus units in several respects. Twitch times-to-peak and half-relaxation times were shorter than normal soleus (Table 6-3), suggesting a degree of neural influence upon these parameters. Maximum tetanic tension was greatly increased in longX-soleus type S units compared to normal soleus type S units. Tetanic tensions of some type S units in longX-soleus were much

larger than in normal soleus (Fig. 6-2C). Fatigue resistance was similar in longX-soleus type S units and normal soleus type S units (Table 6-3, Fig. 6-2D).

In normal soleus, we found that 61% (27/44) of the units exhibited depression of twitch tension following a 100Hz tetanic stimulation of 600ms (post tetanic depression: PTD), compared to only 25% (5/20) of the type S motor units in MG. Following innervation by MG motoneurons, 60% (15/25) of soleus type S units exhibited PTD (43% of all units: 15/35). Chan et al. (1982) saw a slight shift from four of 17 normal soleus units showing PTD, to only one of 32 following x-reinnervation with FHL nerve. Dum et al. (1985) observed a virtually complete conversion of this property following X-reinnervation of soleus by FDL. It is unclear what underlies these differences between laboratories.

In summary, longX-soleus contains some fatiguable fast units not present in control soleus. The number of fast units is less than expected, based upon the proportions of motor unit types in MG. This suggests 'resistance' of soleus muscle fibers to type conversion. The fast units generate little tension, but are otherwise similar to gastrocnemius units of the same type. The small size of fast units, and large size of slow units, may indicate that type S motoneurons capture more muscle fibers (c.f. Lewis et al. 1982), but this interpretation is complicated by the failure of some muscle fibers to convert to fast type. Long X-soleus type S units are similar to control soleus type S units, although speed-related parameters have shifted somewhat in the direction of gastrocnemius type S units. LongX-soleus type S units are

large in relation to both longX-soleus fast units and to normal soleus type S units.

The medX-soleus (n = 14) sample included only one type FR unit, with the rest type S. Type S muscle units at the medX stage were 'slower', with repect to twitch time-to-peak and half-relaxation time than after nine months X-reinnervation (Tables 6-3, 6-7). This suggests that conversion of muscle unit properties is incomplete at this stage. Twitch and tetanic tensions of medX-soleus units were smaller than longX-soleus units, while mean fatigue index was similar.

X-Soleus Muscle Fiber Histochemistry. Normal soleus contains nearly 100% type SO muscle fibers, with a few (<5%) FOG fibers in some muscles (Table 6-5; CHAPTER V; Burke et al., 1974; Edgerton et al. 1980). Long X-soleus contained a variable number of FOG muscle fibers. In longX-soleus muscles we found 23 to 2,076 fibers with high alkali-stable myosin ATPase staining (type II; Engel, 1970). The type II muscle fibers were located throughout the muscle belly. For eight muscles, the mean was 509 type II fibers (3 had >500; 5 had < 500). We found no type II fibers in three of the four medX-soleus muscles. The other muscle contained 389 type FOG fibers. If one assumes 20,000-30,000 muscle fibers in soleus, (Clark, 1931) this translates to less than 11% type II in all cases. This is in agreement with previous studies of X-reinnervated soleus (Chan et al. 1982; Burke, 1980; Gauthier et al. 1983; Edgerton et al. 1978); but see Lewis et al. 1982). Lewis et al. (1982) estimated that a mid-section of soleus samples approximately 88% of the muscle fibers. Thus we may slightly underestimate the number of type II fibers.

Table 6-5. Percent Muscle Fiber Types: Normal and Cross-Reinnervated.

	FG	FOG	SO	N [a]
Normal MG	57	28	15	4
Self-reinnervated MG	54	15	31	5
Normal LG	65	16	18	4
LongX-LG	64	13	23	3
Normal Soleus			100	8
LongX-Soleus		0-11	89-100	8

a. Number of animals.

Table 6-6. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types (LongX).

	X-LG	P	X-SOLEUS	P
Twitch Amplitude [a,b]	F>(R,S)	0.01	NS	0.05
Twitch Time-To-Peak [b]	(F,I,R)<S	0.01	(R,I)<S	0.01
Twitch HRT [b,c]	(F,I,R)<S	0.01	(R,I)<S	0.01
Tetanic Tension [a]	F>(R,S)	0.01	NS	0.05
Fatigue Index	F<I<(R,S)	0.01	(F,I)<(R,S)	0.01

a) The same result was obtained with raw data and data normalized by body weight.
b) Potentiated twitch.
c) HRT = half-relaxation time.

Table 6-7. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types (MedX).

	X-LG	P	X-SOLEUS	P
Twitch Amplitude [a,b]	NS	0.05	NS	0.05
Twitch Time-To-Peak [b]	(F,I,R)<S	0.01	NS	0.05
Twitch HRT [b,c]	(F,I,R)<S	0.01	NS	0.05
Tetanic Tension [a]	F>(R,S)	0.05	NS	0.05
Fatigue Index	F<I<(R,S)	0.01	NS	0.05

a) The same result was obtained with raw data and data normalized by body weight.
b) Potentiated twitch.
c) HRT = half-relaxation time.

Virtually all type II fibers also stained dark for NADH-D (type FOG; Peter et al. 1972). In four of eight muscles we found a small number (21-315) of type FG muscle fibers. The largest number of these were found in the animal with the largest number of type II muscle fibers. Lewis et al. (1982) found 5% type IIb muscle fibers in soleus, X-reinnervated by FDL nerve.

For longX-soleus the proportion of type II muscle fibers was less than the proportion of fast motor unit types. This suggests low innervation ratios for fast units (see below; Lewis et al. 1982), or a mixture of muscle fiber types within single units. Glycogen depletion experiments (Burke, 1980; Dum et al. 1985) suggest that X-reinnervated soleus motor units are homogeneous in type. Gauthier et al. (1983) showed by immunohistochemistry that myosin from X-reinnervated soleus (FDL nerve) was different from normal soleus, but that conventional histochemical methods were insensitive to the change.

All type II muscle fibers also stained dark for a-··· dehydrogenase (Edgerton et al. 1980). In two animals, we observed a number of fibers which stained dark for a- ··· dehydrogenase, which did not stain dark for myosin ATPase (see also Edgerton et al. 1980; Romanul and Van Der Meulen, 1967). Glycolytic metabolism may be a more sensitive indicator of neural influence on soleus muscle fibers than myosin ATPase.

X-Soleus Muscle Fiber Areas and Innervation Ratios. Long X-soleus was innervated by about 60% of the number of motoneurons innervating normal soleus [83 vs. 145; based on (280 normal MG motoneurons - 10% non-contracts)(33% innervate soleus)]. Mean muscle fiber areas in

longX-soleus were reduced (Table 6-8), compared to normal soleus (CHAPTER V). Assuming a normal, or slightly below normal number of muscle fibers, the reduced number of motoneurons requires increased mean innervation ratio. We found longX-soleus muscle fiber types to differ in cross-sectional area, with fiber size increasing in the order FG<FOG<SO. This order is reversed from normal or reinnervated gastrocnemius units (CHAPTER III). Lewis et al. (1982) found no difference in fiber size between type II and type I muscle fibers in two soleus muscle innervated by FDL nerve. The rare type FOG fibers in normal soleus are of similar size to soleus type SO fibers (CHAPTER V).

MedX-soleus was innervated by approximately 36% of the normal number of motoneurons (52 vs. 145; calculated as above; 23% non-contracts). Mean muscle fiber areas were smaller than at nine months. MedX-soleus type SO fibers averaged 1890um^2 and type FOG were 1001um^2. We found no type FG muscle fibers in medX-soleus muscles.

Discussion

This study examined two aspects of the relationship between motoneurons and the muscle fibers they innervate. First, we tested whether reinnervation of mammalian skeletal muscle was selective with respect to motor unit type. Second, we examined the degree to which muscle unit contractile properties and muscle fiber histochemistry were determined by the motoneuron. A third aspect, whether the type of muscle innervated influences the expression of motoneuron electrical properties, will be addressed in the next chapter. The model employed to address these questions was surgical cross-reinnervation of the cat lateral gastrocnemius (LG) and soleus muscles by medial gastrocnemius

Table 6-8. Motor Unit Types, Muscle Fiber Types and Innervation Ratios (LONG-X).

MUSCLE FIBER TYPES

Histochemical Composition

	FG	FOG	SO
X-LG	64	13	23
X-Soleus		0-10	90-100

Observed Mean Fiber Area (um^2)

				ALL
X-LG	4613	2663	2021	3349 [e]
X-Soleus	1117	2038	2647	2647 [f]

EQUIVALENT MOTOR UNIT TYPE

% Of Population

	FF+FI	FR	S	n/N [a]
X-LG	51+5	27	17	93/10
X-Soleus	2+11	20	67	45/10

Est. Number in Pool [d]

				ALL [b]
X-LG	95	46	29	169
X-Soleus	11	17	56	83

CALCULATED VALUES

Relative IR [c]	FF+FI	FR	S
X-LG	1.1	0.5	1.4
X-Soleus			1.0

a. Number of Cells/Number of Animals.
b. X-LG: [280 - (280)(10% non-contracts)] x (.67); X-soleus: [280 - (280)(10% non-contracts)] x (.33).
c. Relative Innervation Ratio = %Muscle Fiber Type/% Motor Unit Type.
d. Estimated as (Total number of motoneurons)(% motor unit type).
e. Weighted average of sum of (%muscle fiber type)(mean area for muscle fiber type).
f. Based on type SO fibers only.
g. Means for each type calculated as weighted average, assuming constant innervation ratio between compartments, and that the proportion of motoneurons innervating each compartment is as normal LG (Weeks and English, 1985).

(MG) motoneurons. We examined whole muscle and motor unit properties at two times after the initial surgery: nine to ten weeks (medX) and nine to eleven months (longX).

Selectivity of Reinnervation

The MG nerve was surgically re-routed into the combined LG- nerve. This gave MG motoneurons a 'choice' between innervating LG, a mixed muscle with all muscle fiber types in proportions similar to normal MG (Ariano et al. 1973; English and Letbetter, 1982b; CHAPTER V), and soleus, an unusual muscle in that it normally contains nearly 100% slow muscle fibers (Ariano et al. 1973; Burke et al. 1974; CHAPTER V). Our data suggest that the observed distribution of motor unit types in the two muscles (X-LG and X-soleus) was not due to selective reinnervation of soleus muscle by MG type S motoneurons.

There are several lines of evidence which collectively suggest this conclusion. First, there was a random assortment of MG axons entering endoneurial tubes leading to LG and soleus. This is indicated by the agreement between the proportion of motoneurons innervating the two muscles, and the number of axons normally supplying LG and soleus. Furthermore, the X-LG motor units were in normal MG proportions, suggesting neural control of motor unit type. Since the X-LG sample represented two-thirds of the sampled units, it seems unlikely that the distribution of motor unit types in the remaining one-third (X-soleus) can be explained by selective reinnervation. To carry this argument one step further, we calculated the number of type S motoneurons required to account for the overall X-reinnervated proportion of type S motor units, and compared this value to the number of type S motoneurons available in

the normal MG sample. This analysis indicated that there were not enough type S motoneurons available in the original pool to account for the observed distribution on the basis of selective reinnervation. We conclude that the X-soleus distribution results from incomplete conversion of muscle fiber types by the MG motoneurons, which represent a normally distributed sample of MG motoneuron types.

Previous studies in which cat soleus muscle was cross-reinnervated with a foreign mixed nerve have shown similar incomplete conversion of soleus whole muscle contractile properties (Buller et al. 1960; Eccles et al. 1962; Burke, 1980; Dum et al. 1979; Chan et al. 1982;), muscle histochemistry (Romanul and Van Der Meulen, 1967; Burke, 1980; Dum et al. 1985b; Gauthier et al. 1983; Chan et al. 1982; but see Lewis et al. 1982), and motor unit type proportions (Burke, 1980; Dum et al. 1979, 1985b; Chan et al. 1982), and contractile properties (Burke, 1980; Dum et al. 1979, 1985b; Chan et al. 1982; Bagust et al. 1981; but see Lewis et al. 1982).

Most studies of competition between nerves for innervation of muscle have given two or more nerves access to one muscle (Hoh, 1975; Frank et al. 1975; Goldring et al. 1981; Ip and Vrbova, 1983). The general trend is for a degree of preference for self-reinnervation (at least for mixed muscle and nerves), when the choice for innervation is narrowed. Brushart and Mesulum (1979) cut the sciatic nerve of rats and observed random reinnervation of muscles. The studies of Hoh (1975) and Goldring et al. (1981) indicated preferential innervation of a mixed muscle (EDL or FDL, respectively) by its own nerve, but that soleus muscle accepted either nerve equally well. Ip and Vrbova (1983) showed that

neuromuscular junctions of rat soleus muscle innervated by soleus nerve matured faster than soleus muscle innervated by EDL nerve. In the present study, MG axons were given a 'choice' between two muscles, neither of which was the original muscle. Our data suggest no selectivity of reinnervation on the basis of motor unit type (see also Miledi and Stephani, 1969).

Relative innervation ratios of types FR and S motor units in self-reinnervated MG, X-LG and X-soleus, suggest that type S motoneurons may be favored in competition for capturing and/or maintaining connections with muscle fibers (see also Bagust et al. 1981; Lewis et al. 1982). Type S advantage could be mediated by differential rate of axonal growth (Lewis et al. 1982), or a type-recognition process, in which slow motoneuron to slow muscle fiber connections are favored and all other combinations of slow and fast axons and muscle fibers are selectively neutral (Ip and Vrbova, 1983; Lewis et al. 1982). There are indications of muscle fiber type recognition by axons during mammalian development (Thompson et al. 1984, see also O'Donovan, 1985). The motor unit type proportions in medX-LG and medX-soleus are consistent with either earlier arrival of type S axons, or incomplete conversion of muscle fibers by fast motoneurons (relative to longX-soleus).

We conclude that cross-reinnervation of LG and soleus muscle by MG motoneurons was non-selective. The X-soleus distribution is most parsimonously explained by failure of soleus muscle fibers to undergo conversion of type, despite innervation by the full complement of MG motoneuron types. There may be a slight advantage for type S units and disadvantage for type FR units during reinnervation of MG, soleus and

LG, by MG motoneurons. This advantage could be due to differences in rate of axonal outgrowth, ability of axons to establish and/or maintain connections with muscle fibers, or ability of motoneurons to alter muscle fiber properties.

Motoneuron Influence on Muscle Properties

The normal proportions of motor unit types are re-established after long-term self-reinnervation of MG, or long-term cross-reinnervation of LG muscle by MG motoneurons. As mentioned above, this is not true for soleus muscle reinnervated by MG motoneurons. X-soleus contains predominately type S motor units, although approximately one-third of the units are fast. This suggests that motor unit type distribution can be dictated completely by motoneurons in the gastrocnemii, but that soleus muscle fibers resist conversion. Our data suggest that incomplete conversion of soleus whole muscle properties reflects incomplete conversion of individual fibers, as well as the reduced size of fast motor units. Muscle histochemistry of longX-LG suggests that the LG_m compartment (English and Letbetter, 1982a,b) may also 'resist' motoneuron-induced alteration of properties.

Motor unit type is defined by twitch time-to-peak (or 'sag') and fatigue resistance, with force generation an important corollary, as motor unit recruitment appears to be organized by unit force (Zajac and Faden, 1985). The data for self-reinnervated MG and X-LG units show that speed- and fatigue-related properties show complete recovery to normal levels, suggesting that these parameters are highly influenced by neural input. Neural input regulates speed-related properties in X-soleus, but to a lesser extent than in the gastrocnemii. Unlike normal soleus, some

X-soleus units have short times-to-peak and half-relaxation times. Also, X-soleus type S units are faster than normal soleus units, and similar to gastrocnemius type S units in this regard. Most X-soleus units remain highly fatigue-resistant, and stain intensely in histochemical stains for oxidative enzymes, suggesting that these properties are also controlled, to a great extent, by the intrinsic genetic program of the muscle fiber (Edgerton et al. 1980).

Twitch time-to-peak is influenced by muscle fiber myosin type, activation of regulatory proteins and reuptake of intracellular calcium by the sarcoplasmic reticulum, as well as muscle length and compliance (Close, 1972; Lewis et al. 1982). The incomplete conversion of X-soleus twitch speeds is in part reflected in the lack of conversion of histochemically-determined myosin ATPase. Gauthier et al. (1983) have shown that X-soleus myosin is different from normal soleus, but that standard histochemical procedures are insufficiently sensitive to detect this. Half-relaxation time more closely reflects the active state dynamics. This property is also incompletely converted. In X-LG, speed- and fatigue-related properties recover by ten weeks post-operative. Alteration of twitch speed in X-soleus is less complete at ten weeks than at ten months.

Both fatigue resistance and twitch speed can be altered by chronic electrical stimulation, although conversion of fast units to slow is easier to demonstrate than the reverse (reviewed in Salmons and Henrickson, 1981; Pette, 1984). This suggests that much of the neural-induced muscle fiber alterations could be due to altered amounts or pattern of activity (Dum et al. 1985a,b; O'Donovan et al. 1985;

Pette, 1984; Lomo et al. 1974, 1980). The incomplete conversion of soleus could be due to an intrinsic lack of phenotypic plasticity of the muscle fibers. Alternatively, it is possible that an activity threshold for muscle fiber conversion was not reached due to the different mechanical arrangement of soleus from the gastrocnemii (soleus crosses only one joint, has less pennate fiber arrangement, has longer muscle fibers). It is not possible to differentiate these possible mechanisms with the present paradigm.

Muscle unit force also appears to be neurally influenced. Following nine months self-reinnervation, MG types S and FR units completely recover tetanic tension levels (CHAPTER III). In contrast the type FF motor units did not fully recover. The degree of recovery followed the recovery of muscle fiber area, with little change in innervation ratios (CHAPTERS III, IV). X-LG motor units recovered tetanic tension and muscle fiber areas completely after nine months recovery time. It is unclear what the difference between type FF units was for the self-and cross-reinnervated gastrocnemii. At ten weeks post-operative, X-LG mean muscle unit force was lower than after ten months. In all cases, tensions decrease in the order FF>FR>S (muscle fiber area: FG>FOG>SO). Muscle fiber area and motor unit tension are responsive to the degree of activity and the load against which contractions occur (reviewed in Saltin and Gollnick, 1984). Perhaps mechanical factors resulted in X-LG type FF units being recruited in opposition to greater loads than self-reinnervated MG type FF units.

LongX-soleus fast units generated little tension (Lewis et al. 1982). This was due to low innervation ratio and small muscle fiber

area. The low innervation ratios for X-soleus fast units may reflect competition between fast and slow motoneurons in capturing and maintaining connections with muscle fibers. X-soleus type S units generate higher than normal tension. The low number of innervating axons suggests that increased innervation ratio is largely responsible for the supernormal motor unit size. Type SO muscle fibers were smaller in X-soleus than in normal soleus, and more similar in size to type SO fibers in the gastrocnemii. This suggests a strong neural influence on type SO muscle fiber area. X-soleus muscle fibers increased in size in the order FG<FOG<SO, opposite of normal and self-reinnervated MG or cross-reinneravated LG. This indicates that those soleus muscle fibers which are converted by MG fast motoneurons, do not respond to neural influence in the same manner as gastrocnemius muscle fibers.

The general pattern is that muscle unit speed-, fatigue-, and force-related properties are highly influenced by motoneurons in MG or LG, but adult soleus muscle fibers do not exhibit the same degree of phenotypic plasticity as muscle fibers in the mixed muscles (Burke, 1980; Dum et al. 1985b). Soleus muscle fibers respond differently to neural input, resulting in less alteration of muscle fiber type, twitch speed, fatigue resistance and tetanic force.

During development or evolution, two possible ways to express specialization of properties of a muscle, or part of a muscle, would be direct alteration of the intrinsic muscle genetic program, or indirectly by modification of the motoneuron pool, which then regulates muscle phenotype. Studies of adult regeneration demonstrate an important role for the motoneuron in regulating muscle properties. The results for cat

soleus (and the LGm compartment of LG: see Results, this chapter) indicate that there are limits to neural regulation. In this case, the intrinsic genetic program appears to dominate phenotypic regulation. There is also evidence for neural-independent type specialization of muscle fibers during development (reviewed in O'Donovan, 1985).

The question remains as to whether the combination of non-selective reinnervation and 'resistance' to change by soleus muscle fibers results in a mismatch between motoneuron electrical properties and muscle unit contractile properties, or whether motoneurons exhibit plasticity in expression of their electrical properties. The next chapter addresses this question.

CHAPTER VII
CROSS-REINNERVATION OF LG AND SOLEUS MUSCLES BY MG MOTOR NERVE: MUSCLE INFLUENCE UPON MOTONEURONS

Introduction

The preceding chapter demonstrated that cross-reinnervation of the LG and soleus muscles by MG nerve was non-selective, with respect to motor unit type. MG motoneurons influenced the phenotypic expression of LG muscle fibers in a similar manner to their effect on MG muscle fibers. In contrast, soleus muscle fibers 'resisted' neural influence from MG motoneurons, thereby remaining predominately slow in properties. This paper addresses two aspects of influence of the periphery on motoneurons. First, is functional connection to muscle a sufficient condition for recovery of motoneuron electrical properties, following nerve section and repair (CHAPTERS III, IV)? Second, do motoneuron electrical properties depend upon the particular muscle innervated? That is, does soleus muscle fiber 'resistance' to type-conversion result in a mis-match between motoneuron electrical properties and muscle unit contractile properties, or do motoneurons alter their properties as well?

Several studies have shown that the pattern of activity of a cross-reinnervated muscle is that of the muscle originally innervated by the foreign nerve (Sperry, 1945; Brinkman *et al.* 1978; O'Donovan *et al.* 1985;), although Cohen (1978) did find some evidence for respecification of the neural activity pattern. This observation suggested to many

workers that neural properties were unaltered following regeneration into a foreign muscle. Kuno et al. (1974b) reached a similar conclusion based upon measures of AHP duration and amplitude, action potential overshoot, and resting potential of self-regenerated and cross-regenerated flexor digitorum longus (FDL) and soleus motoneurons at various times up to 150 days post-operative (see also Chan et al. 1982, for axonal conduction velocity).

In contrast, other studies have reported changes in axonal conduction velocity (Lewis et al. 1978; Lewis et al. 1982; Burke, 1980; Dum et al. 1985a,b), or AHP duration (Burke, 1980; Dum et al. 1985a,b) following innervation of a foreign muscle. Numerous studies by Kuno and colleagues (Czeh et al. 1978; Gallego et al. 1978, 1979; Huiszar et al. 1977) have emphasized an influence of muscle on expression of motoneuron AHP duration. Tomanek and Tipton (1967) reported an influence of muscle mass on axon diameter (see also Edds, 1950; Walsh et al. 1978).

Normally, there is a strong relationship between motoneuron properties and muscle unit contractile properties in cat MG (Fleshman et al. 1981; Burke, 1981; Zengel et al. 1985; CHAPTER III), LG (CHAPTER V) and soleus (Burke, 1981; Cope et al. 1983; Huiszar et al. 1977; CHAPTER V). Following nerve section, motoneurons dedifferentiate with respect to electrical properties (Kuno et al. 1974a; Gustafsson and Pinter, 1984; CHAPTER IV), and for MG motoneurons, motoneuron types are no longer recognizable (CHAPTER IV). By nine months post-operative, MG motoneurons self-regenerated into MG muscle exhibit normal motoneuron electrical properties (CHAPTER IV).

This study utilized the cross-reinnervation model to test whether the expression of motoneuron electrical properties is influenced by the particular muscle innervated. The MG nerve was surgically re-routed into the combined lateral gastrocnemius-soleus (LG-S) nerve (see CHAPTER VI).

Results

Non-Contracts

After nine-to-11 months X-reinnervation, 10% (16/154) of the motoneurons sampled did not elicit muscle tension responses. In self-reinnervation experiments, 10% of motoneurons did not elicit muscle tension (CHAPTER III; Kuno *et al.* 1974b). We refer to these cells as non-contracts, as we cannot rule out that they may have innervated intrafusal muscle fibers (Gregory *et al.* 1982), or had been injured in the final dissection. Mean values and individual data for these cells are presented in Table 7-1.

There were no differences between mean values for electrical properties of the non-contracts in the longX-reinnervated and the self-reinnervated paradigms. In addition, non-contracts at the ten-week X-reinnervation stage did not differ in electrical properties from non-contracts in the other models (Table 7-1). Non-contracts in all models differed from the innervated populations in rheobase, axonal conduction velocity and rheobase/input resistance (all lower in non-contracts), as well as input resistance (higher in non-contracts). Mean AHP half-decay time was similar to the innervated population. Non-contracts' properties overlap with those of innervated type S units (MG, X-LG, or X-soleus) for all parameters but AHP half-decay time,

Table 7-1. Data for Non-Contracts (LongX).

CELL	RHEO [a]	RN [b]	RHEORN [c]	HALFTIME [d]	C.V. [e]	PSP [f]
1	5	2.5	2.0	31	41	0.4
2	7	2.7	2.5	27	70	0.6
3	4	1.9	2.1	34	45	3.4
4	5	0.8	6.6	22	72	0.3
5	3	0.7	4.4	30	53	0.5
6	4	2.3	1.5	36	74	0.4
7	3	2.5	1.0	36	64	0.6
8	7	X	X	46	89	0.5
9	2	X	X	46	41	0.1
10	5	1.5	3.4	21	72	0.5
11	9	1.3	6.3	28	80	1.9
12	14	0.7	19.3	17	75	X
13	19	1.2	16.9	14	85	0.6
14	9	0.9	10.6	20	83	0
15	4	2.0	2.8	32	86	0.8
16	8	2.8	1.9	25	X	X

MEAN VALUES ±SE (NUMBER OF UNITS)

	NON-CONTRACTS	X-REGENERATED	CONTROL
RHEOBASE (nA)	7±1 (16)	10±1 (133)	14±1 (147)
RN (Mohm)	2±0 (14)	1±0 (111)	1±0 (117)
RHEO/RN (nA/Mohm)	6±2 (14)	11±1 (111)	21±2 (116)
HALFTIME (ms)	28±2 (16)	30±1 (130)	28±1 (111)
AXONAL C.V. (m/s)	68±5 (15)	87±1 (126)	94±1 (118)

a) RHEO = rheobase
b) RN = input resistance
c) RHEO/RN = rheobase/input resistance
d) HALFTIME = AHP half-decay time
e) C.V. = conduction velocity
f) PSP = composite monosynaptic group Ia EPSP from LG-S nerve (innervating MG)

which is more typical of fast motoneurons, and axonal conduction velocity, which is lower in non-contracts (CHAPTER IV).

As in the self-reinnervation paradigm (CHAPTER III), most longX non-contracts (12/16 = 75%) were outside the normal range for fast or slow motoneurons for the ratio AHP half-decay time: axonal conduction velocity (Fig. 7-2B). Non-contracts had lower mean axonal conduction velocity and a narrower range of AHP half-decay times than type S motoneurons. A similar pattern can be seen for medX non-contracts (Fig. 7-2A). The relationship between rheobase and input resistance for non-contracts overlaps with type S motoneurons (Fig. 7-3B for longX; Fig. 7-3A for medX). Non-contracts at all stages were similar in properties to axotomized motoneurons (Kuno et al. 1974a; Gustafsson, 1974; Gustafsson and Pinter, 1984; CHAPTER IV). These data are consistent with the notion that functional connection to muscle is necessary for expression of mature motoneuron electrical properties (CHAPTER IV).

Properties of Cross-Innervated Motor Units

Motoneuron electrical properties: MG --> LG. The primary control population for X-regenerated MG motoneuron properties was nine-month self-regenerated MG (CHAPTER III) to control for any changes due to nerve section and regrowth per se.

MG motoneurons innervating LG muscle for nine months were similar in electrical properties to self-regenerated MG motoneurons (Table 7-2,

Table 7-2. Motoneuron Electrical Properties: Cross-Reinnervation.[a,b]

	FF	FI	FR	S	ALL
RHEOBASE (nA)					
SELF-REGENERATED	24±1 (33)	18±3 (3)	11±1 (18)	6±1 (19)	15±1 (73)
LONGX (ALL)	18±1 (38)*	11±2 (8)	9±1 (33)	5±1 (35)	10±1 (114)**
X-LG	18±1 (37)*	12±2 (4)	8±1 (22)	4±1 (10)	13±1 (73)
X-SOLEUS	35 (1)	12±3 (4)	9±1 (11)	5±1 (25)	8±1 (41)**
MEDX (ALL)	16±1 (14)	17±4 (11)	12±1 (7)	5±1 (20)	12±1 (52)
X-LG	16±1 (14)	17±4 (11)	12±2 (6)	5±2 (7)	13±2 (38)
X-SOLEUS			12 (1)	5±1 (13)	6±1 (14)**
INPUT RESISTANCE (Mohms)					
SELF-REGENERATED	0.7±0 (22)	0.7±0 (2)	1.2±0 (10)	1.3±0 (11)	1.0±0 (117)
LONGX (ALL)	0.9±0 (33)	0.9±0 (7)	1.5±0 (28)	2.0±0 (27)	1.4±0 (95)
X-LG	0.9±0 (32)*	0.8±0 (3)	1.6±0 (20)	2.1±0 (9)*	1.3±0 (64)
X-SOLEUS	1.4 (1)	1.1±0 (4)	1.2±0 (8)	2.1±0 (18)*	1.7±0 (31)
MEDX (ALL)	0.6±0 (13)	0.7±0 (11)	0.8±0 (7)**	1.4±0 (19)	0.8±0 (50)
X-LG	0.6±0 (13)	0.7±0 (11)	0.7±0 (6)*	1.2±0 (7)	0.8±0 (37)
X-SOLEUS			1.2 (1)	1.5±0 (12)	1.5±0 (13)
RHEOBASE/INPUT RESISTANCE (nA/Mohms)					
SELF-REGENERATED	40±4 (22)	33±12 (2)	10±2 (10)	6±1 (11)	23±3 (45)
LONGX (ALL)	24±4 (33)	12±1 (7)	7±1 (28)	2±0 (27)	11±1 (95)**
X-LG	25±4 (32)	13±2 (3)	7±1 (20)	2±0 (9)*	15±2 (64)
X-SOLEUS	25 (1)	11±2 (4)	10±3 (8)	3±0 (18)**	6±1 (31)**
MEDX (ALL)	30±4 (13)*	42±20 (11)	18±4 (7)**	4±1 (19)	24±4 (49)
X-LG	30±4 (13)*	42±20 (11)	19±5 (6)**	4±0 (7)	27±6 (37)
X-SOLEUS			10 (1)	4±1 (12)	5±1 (12)**
AHP HALF-DECAY TIME (ms)					
SELF-REGENERATED	20±1 (33)	23±1 (3)	24±2 (18)	44±3 (19)	27±1 (73)
LONGX (ALL)	22±1 (37)	24±2 (8)	24±1 (33)	46±3 (32)	30±1 (110)
X-LG	21±1 (36)	22±1 (4)	25±1 (22)	47±4 (9)	26±1 (71)
X-SOLEUS	50 (1)*	25±5 (4)	23±2 (11)	80±2 (23)	37±3 (39)**
MEDX (ALL)	18±1 (14)	21±1 (9)	22±2 (7)	40±3 (20)	28±2 (50)
X-LG	18±1 (14)	21±1 (9)	23±3 (6)	41±5 (7)	24±2 (36)
X-SOLEUS			16 (1)	40±4 (13)	38±4 (14)**
AXONAL CONDUCTION VELOCITY (ms)					
SELF-REGENERATED	96±2 (33)	94±5 (3)	96±2 (18)	84±2 (19)	90±1 (73)
LONGX (ALL)	97±2 (36)	90±6 (5)	91±2 (30)	82±2 (35)	87±1 (106)
X-LG	97±2 (35)	89±8 (3)	92±2 (20)	88±3 (12)	94±2 (70)
X-SOLEUS	93 (1)	87 (2)	90±3 (10)	80±2 (23)	83±2 (36)
MEDX (ALL)	78±3 (15)**	78±2 (11)	78±3 (7)**	68±2 (20)**	76±2 (52)**
X-LG	78±3 (15)**	78±2 (11)	77±3 (6)**	68±3 (7)**	76±2 (39)**
X-SOLEUS			79 (1)	68±2 (13)**	69±2 (13)**

a. Means ± SE (number of units).
b. * = Significant difference from SELF-REGENERATED ($p<0.05$); ** ($p<0.01$).

Figs. 7-1,7-5). The only exception was mean rheobase of type FF motoneurons, which was lower in longX-LG than in self-regenerated MG. There was a general trend for MG motoneurons which innervated LG to have lower rheobase (Fig 7-1A). The input resistance distribution for all motor unit types was shifted slightly towards higher values (Table 7-2). Motoneuron electrical properties differed between motor unit types, as in self-regenerated MG motor units (Table 7-3).

Both fast and slow MG motoneurons innervating LG muscle had a greater range of axonal conduction velocities than in self-regenerated MG (Fig. 7-3D). The distribution for AHP half-decay time was similar for self-regenerated MG motoneurons and MG motoneurons which innervated LG muscle (Fig. 7-1C). AHP half-decay time and axonal conduction velocity were negatively correlated for MG motoneurons innervating LG ($r = -0.38$, $p<0.003$).

The distribution of the ratio AHP half-decay time: axonal conduction velocity is shown in Fig. 7-2. MG motoneurons which innervated LG muscle fell within the normal range (CHAPTER III) 67% of the time (42/63; Fig. 2B). Sixty-seven percent of fast (FF+FI+FR) motoneurons (35/52) were in the normal fast range, and 64% of slow motoneurons (7/11) were in the normal slow range. Most motoneurons outside the normal range for AHP half-decay time: axonal conduction velocity exhibited the extremes of the range of axonal conduction velocities, for their type. Following 150 days self- or x-reinnervation, Kuno and coworkers (1974b) found 50% of soleus motoneurons within the normal range for this relationship.

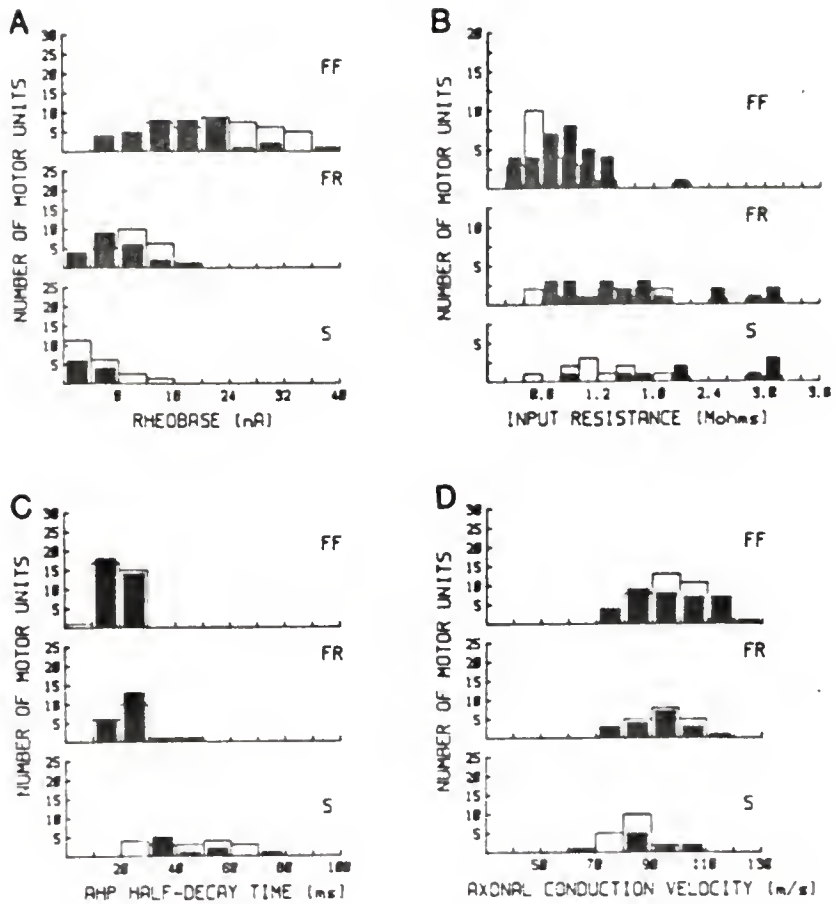

Figure 7-1. Frequency histograms for electrical properties of self-regenerated MG motoneurons (unfilled), and MG motoneurons which innervated longX-LG. (A) rheobase (B) input resistance (C) AHP half-decay time (D) axonal conduction velocity. See text for explanation.

in normal and self-regenerated MG, motoneurons segregated by motor unit type on the basis of the ratio rheobase:input resistance (CHAPTER III). The correlation between log rheobase and input resistance was -0.53 overall, with no significant relationship within motor unit types (similar to unoperated control and self-regenerated MG; CHAPTER III).

For longX-LG, motoneuron type (determined from electrical properties: Zengel et al. 1985; CHAPTERS III,IV,V) correctly predicted motor unit type (determined from contractile properties) in 74% of cases (45/61). This was lower than in unoperated control MG or self-regenerated MG, reflecting greater variability in distributions for individual parameters (Fig. 7-3). AHP accurately separated fast and slow in 96% of motor units (69/72; fast AHP half-decay time <30ms; slow AHP > 30ms; Zengel et al. 1985), similar to normal and self-regenerated MG motoneurons. The decreased predictive value of motoneuron type in longX-LG motor units was due to decreased rheobase/input resistance in all motoneurons (Table 7-2, Fig. 7-3B), which resulted in the misclassification of several motoneurons from type FF motor units, which had values for this ratio of less than 18.

We conclude that MG motoneurons express motoneuron electrical properties similarly after long-term reinnervation of a foreign mixed muscle (LG), or MG muscle. While mean values for motoneuron electrical properties did not differ statistically from self-regenerated MG, MG motoneurons which innervated LG muscle may not express motoneuron electrical properties exactly as when reinnervating MG muscle.

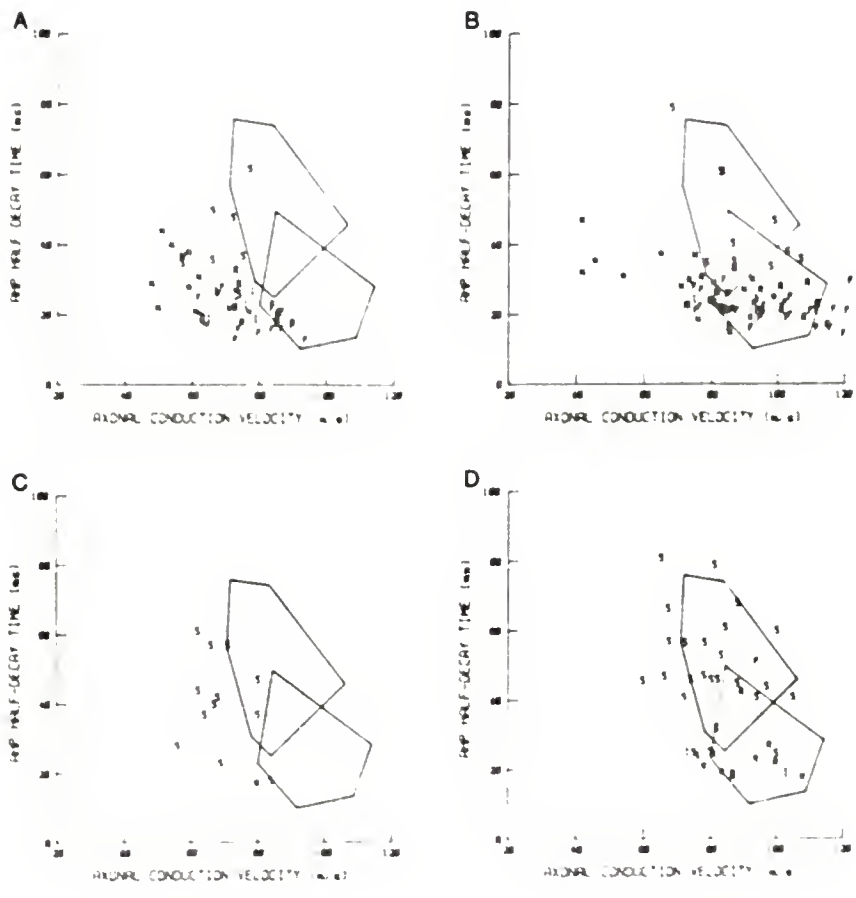

Figure 7-2. Relationship between AHP half-decay time and axonal conduction velocity in MG which innervated LG or soleus. Symbols as in Fig. 7-3. (A) longX-LG (B) medX-LG (C) longX-soleus (D) medX-soleus. area). Solid lines outline the distribution of slow (upper) and fast motoneurons in normal MG (CHAPTER III).

Electrical properties of medX-LG motoneurons are seen in Table 7-2. Mean values for all parameters except axonal conduction velocity (lower at medX stage) were similar to longX-LG motoneurons. Most medX-LG motoneurons were outside the normal range for the ratio AHP half-decay time: axonal conduction velocity (Fig. 7-2A). Fast and slow motoneurons segregated, but axonal conduction velocities were shifted to lower values. MedX motoneurons also segregated by motor unit type with respect to the ratio rheobase:input resistance (Fig. 7-3A). AHP half-decay time predicted fast vs. slow motor units in 94% (34/36) of cases. Motoneuron type correctly predicted motor unit type in 67% (18/27) of units.

Relationships between motoneuron electrical properties and muscle unit contractile properties: MG --> LG. As mentioned above, motoneuron type was an accurate predictor of motor unit type in longX-LG. In longX-LG, the strongest correlation between an individual motoneuron electrical property and a muscle unit contractile property was between AHP half-decay time and twitch time-to-peak ($r = 0.69$, $p<0.0001$). There were no significant correlations between these parameters within motor unit types. No motoneuron electrical property accounts for more than 50% of the variation of any single muscle unit contractile property. This is similar to unoperated control MG or LG, as well as self-reinnervated MG (CHAPTERS III, IV; Zengel et al. 1985; see also Kuno et al. 1974b). For medX-LG, this correlation was 0.70 ($p<0.0001$) overall, with no significant relationship within any motor unit type.

There was a significant overall correlation between axonal conduction velocity and twitch time-to-peak in longX-LG ($r = -0.38$, $p<0.003$). This was similar to unoperated control MG and LG, as well as

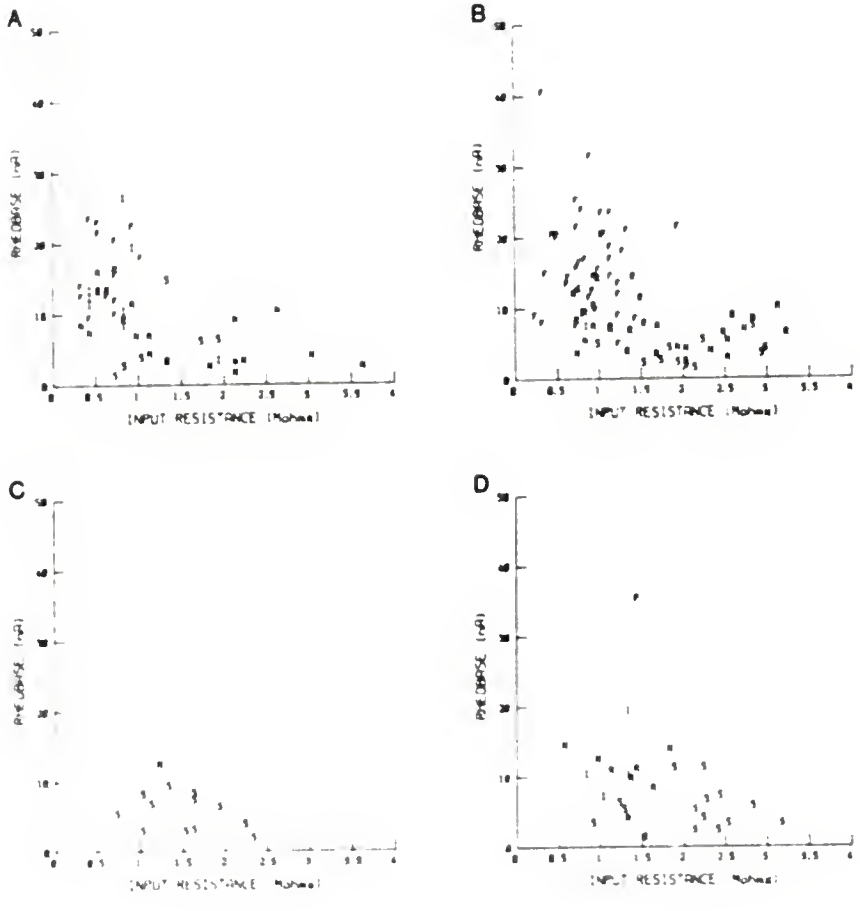

Figure 7-3. Relationships between rheobase and input resistance in MG motoneurons which innervate LG or soleus. F= FF units, I=FI, R=FR, S=S units, N = non-contracts. (A) longX-LG (B) medX-LG (C) longX-soleus (D) medX-soleus.

self-reinnervated MG (CHAPTERS III,IV; see also Dum et al. 1985a). This relationship was also significant at ten weeks X-reinnervation ($r = -0.42$, $p<0.008$). For longX-LG, axonal conduction velocity was significantly correlated with twitch tension (0.35, $p<0.003$) and tetanic tension (0.43, $p<0.0002$) overall, but not within any motor unit type (CHAPTERS III,IV; Zengel et al. 1985). For medX-LG the correlation between axonal conduction velocity and twitch tension was non-significant. There was a weak relationship between axonal conduction velocity and tetanic tension ($r =-0.33$, $p<0.04$). There were no significant correlations within any motor unit type.

We conclude that relationships between motoneuron and muscle unit properties are of similar quality and strength for MG motoneurons innervating MG or LG muscle.

Motoneuron electrical properties: MG --> soleus. Overall mean rheobase and rheobase/input resistance were significantly lower in MG motoneurons which innervated soleus than in self-regenerated MG motoneurons (Table 7-2). Mean AHP half-decay time was longer than that of self-regenerated MG motoneurons (Kuno et al. 1974b; Burke, 1980; Dum et al. 1985; Goldring et al. 1981). MG motoneurons which innervated soleus muscle had lower mean axonal conduction velocity and greater mean input resistance than self-regenerated MG motoneurons, although these differences did not reach statistical significance (Table 2). All of these differences in overall mean values are in the direction of motoneuron properties of normal soleus motoneurons (or type S MG motoneurons; CHAPTERS III,V), in agreement with the predominance of type S units in longX-soleus. Lewis and coworkers (Lewis et al. 1978;

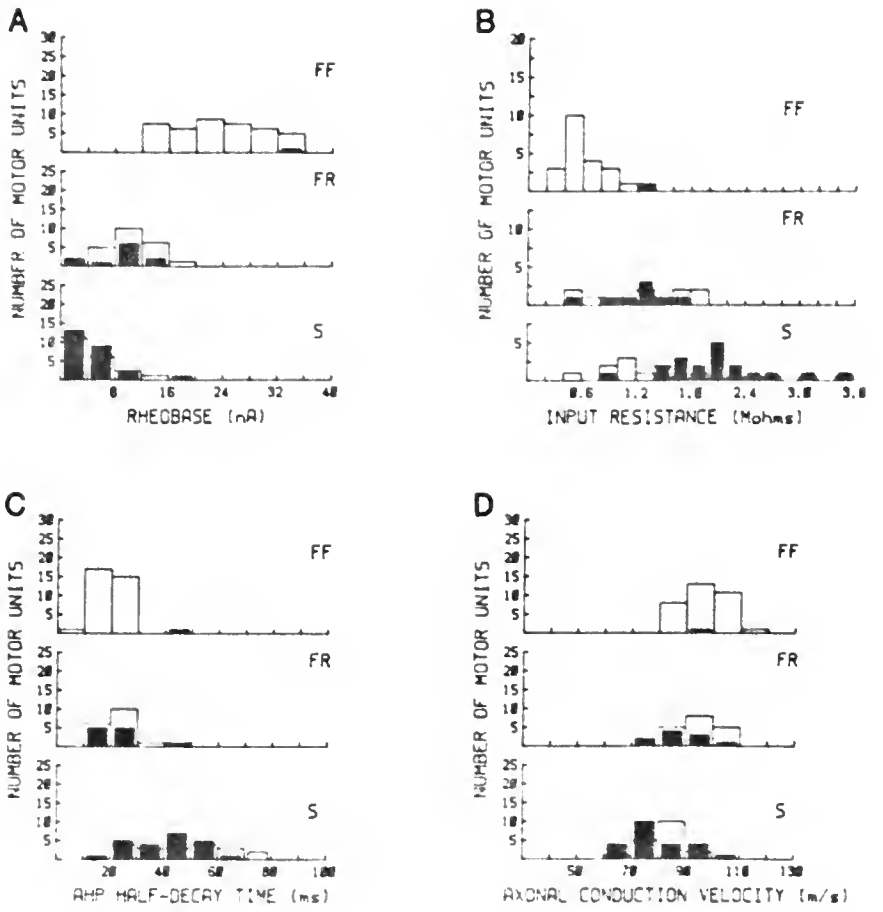

Figure 7-4. Frequency histograms for electrical properties of self-regenerated MG motoneurons (unfilled), and MG motoneurons which innervated longX-soleus. (A) rheobase (B) input resistance (C) AHP half-decay time (D) axonal conduction velocity. See text for explanation.

Lewis et al. 1982) reported significantly lower conduction velocities for FDL or FHL axons innervating soleus. Several other studies have found no statistical differences in axonal conduction velocity of foreign nerves innervating soleus (Burke, 1980; Dum et al. 1985; Chan et al. 1982; Kuno et al. 1974b).

The only significant difference within a motor unit type was for rheobase/input resistance, which was lower for type S motoneurons which innervated longX-soleus than in self-regenerated MG (Table 7-2). This suggests that motoneuron properties vary according to motor unit type for MG motoneurons which innervated soleus, MG (CHAPTER III) or LG (this chapter; see Table 7-3). The electrical properties of medX-soleus motoneurons showed a similar pattern to that of longX-soleus. Mean values for all parameters except axonal conduction velocity (lower in medX-soleus), reached longX levels by ten weeks (Tables 7-2, 7-4; Fig. 7-5)

Figure 7-2D shows the relationship between AHP half-decay time and axonal conduction velocity, for MG motoneurons which innervated longX-soleus muscle. Axonal conduction velocity and AHP half-decay time were negatively correlated ($r = -0.41$, $p<0.01$), in longX-soleus (MG) motoneurons. Slightly more than half of the motoneurons were within the normal MG range for this ratio (59% = 23/39). For fast units, 77% were located within the normal fast range, but only 50% of slow motoneurons were within the normal range for slow motoneurons.

If a MG fast motoneuron was able to convert soleus muscle fibers to fast type, the motoneuron's electrical properties were typical for that motor unit type in MG. In particular, the ratio AHP half-decay time:

Table 7-3. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types (LONGX).

	X-LG	P	X-SOLEUS	P
Axonal Conduction Velocity	NS	0.05	NS	0.05
Rheobase	F>(R,S)	0.01	F>I>(R,S)	0.01
			F>I>R>S	0.01
Input Resistance	F<(R,S)	0.01	I<S	0.01
Rheobase/Input Resistance	F>(R,S)	0.01	F>(R,S)	0.01
AHP Half-Decay Time	(F,I,R)<S	0.01	R<S	0.01
			(I,R)<S	0.05

Table 7-4. Results of Tukey's Studentized Range Test: Significance of Differences Between Motor Unit Types (MEDX).

	X-LG	P	X-SOLEUS	P
Axonal Conduction Velocity	NS	0.05	NS	0.05
Rheobase	I>S	0.05	R>S	0.01
Input Resistance	F<S	0.01	NS	0.05
Rheobase/Input Resistance	NS	0.05	R>S	0.05
AHP Half-Decay Time	(F,I,R)<S	0.01	NS	0.05

axonal conduction velocity was typical of that type. Thus within fast motor units, the relationship between AHP half-decay time and axonal conduction velocity was similar for MG motoneurons which innervated soleus, LG or MG (CHAPTER III).

Of the slow motoneurons outside the normal range for AHP half-decay time: axonal conduction velocity, most (8/13) fell within the normal range for soleus motoneurons (lower axonal conduction velocity combined with longer AHP half-decay time: Fig. 5-5B). This suggests that the relationship between these parameters is different between slow MG motoneurons which innervated MG or LG and soleus.

After ten weeks X-reinnervation, MG motoneurons which innervated soleus segregated into fast and slow, on the basis of AHP half-decay time and axonal conduction velocity (Fig. 7-2C). The low axonal conduction velocities shifted values to the left of normal MG (and to the left of medX-LG motoneurons; Fig. 7-2A). For medX-soleus, these two variables were correlated across all units ($r = 0.55$, $p<0.05$), but not within any motor unit type. AHP half-decay time accurately distinguished fast vs. slow for 79% (11/14) of units.

The relationship between rheobase and input resistance for MG motoneurons which innervated longX-soleus is shown in Fig. 7-3D. Motoneurons segregate by motor unit type, as in normal and self-regenerated MG (CHAPTER III), and MG motoneurons which innervated LG muscle (Fig. 7-3A,B). Log rheobase and input resistance were negatively correlated across all units ($r = -0.40$, $p<0.02$). There was no relationship between these variables within motor unit types. A similar

pattern was seen after ten weeks x-reinnervation of soleus by MG motoneurons (Fig. 7-3C).

Motoneuron type accurately predicted motor unit type in 93% (25/27) of units in longX-soleus (100% for medX-soleus). This indicates a particularly tight relationship between motoneuron and muscle unit properties in X-soleus. The altered relationship between AHP half-decay time and axonal conduction velocity suggests that the relationship between motoneuron electrical properties and muscle unit type is qualitatively different for MG motoneurons which innervate soleus, than for MG motoneurons innervating MG or LG. Those motoneurons which converted their muscle fibers to the fast type, exhibit electrical properties typical of that type in normal or self-regenerated MG. Those motoneurons which did not induce motor unit type conversion in their muscle unit did not attain their original properties, but had electrical properties typical of motoneurons of type S motor units in MG, and in some cases similar to type S units of normal soleus.

Relationship between individual motoneuron electrical properties and muscle unit contractile properties: MG --> soleus. The relationship between motoneuron type and motor unit type was particularly strong in longX-soleus. The strongest correlation between a single motoneuron electrical property and a single muscle unit contractile property was again that between AHP half-decay time and twitch time-to-peak ($r = 0.65$, $p<0.0001$). This relationship was also significant among type S motor units ($r = 0.44$, $p<0.03$). This was also the strongest relationship in normal MG (CHAPTER III; Zengel et al. 1985), as well as normal soleus (CHAPTER V; Huiszar et al. 1977).

The correlation between axonal conduction velocity and twitch time-to-peak for longX-soleus was -0.38 ($p<0.02$; Lewis *et al.* 1982). This relationship did not hold within motor unit types. There were no significant correlations between axonal conduction velocity and twitch or tetanic tension. The pattern observed for medX-soleus motor units was similar to that of longX-soleus.

Discussion

In the preceding chapter we demonstrated that cross-reinnervation of LG and soleus muscles by MG nerve was not selective with repect to motor unit type. We inferred that both soleus and LG muscle receive a full complement of all motoneuron types, in normal MG proportions. The motor unit type distribution, and mean values for contractile properties, were re-established to a similar degree in cross-reinnervated LG, and self-reinnervated MG. In contrast, soleus muscle fibers 'resisted' the influence of the MG nerve. In the present study, we tested whether the type of muscle innervated influences the expression of motoneuron electrical properties. That is, does soleus muscle fiber's resistance' to neural influence result in a disruption of the normal close match-up between motoneuron electrical properties and muscle unit contractile properties (CHAPTERS III,IV,V; Zengel *et al.* 1985)? The model employed to address these questions was surgical cross-reinnervation of the cat lateral gastrocnemius (LG) and soleus muscles by medial gastrocnemius (MG) motoneurons. Motor unit properties were examined at two times after the initial surgery: nine to ten weeks (medX) and nine-to-11 months (longX).

In normal MG (CHAPTER III; Fleshman et al. 1981; Zengel et al. 1985; Burke, 1981; Stuart and Enoka, 1983), LG and soleus (CHAPTER V), there is a close relationship between motoneuron electrical properties and motor unit type. Section of the MG nerve leads to dedifferentiation of motoneuron electrical properties (Kuno et al. 1974a; Gustafsson and Pinter, 1984; CHAPTER IV), and motoneuron types cannot be recognized (CHAPTER IV). Self-regenerated motoneuron electrical properties recover gradually with time after reinnervation of MG (CHAPTER IV). After nine months, self-regenerated MG motoneuron electrical properties are indistinguishable from those of normal MG motoneurons (CHAPTER III). Motor unit types are first recognized at nine to ten weeks post-operative, before motoneuron electrical properties reached mature levels (CHAPTER IV).

In these experiments, some motoneurons did not elicit muscle contraction in MG muscle (non-contracts). These cells did not show recovery of motoneuron electrical properties from the axotomized state (CHAPTER IV). Thus functional connection to muscle appears to be a necessary condition for recovery of motoneuron electrical properties, and for long-term self-regeneration, appears sufficient (CHAPTER III). We therefore asked whether the particular muscle innervated influenced the expression of motoneuron electrical properties in MG motoneurons, i.e. is functional connection always a sufficient condition for recovery of normal motoneuron properties.

By nine to eleven months post-operative, MG motoneurons which innervate LG muscle show recovery of mean values for all motoneuron electrical properties to self-regenerated levels. There was more scatter

in the distributions of these parameters (especially axonal conduction velocity), thus relationships between motoneuron properties were not exactly as in normal MG motoneurons. As a result, motoneuron type was slightly less effective in predicting motor unit type within the fast motor units (74% agreement vs. 84% in self-reinnervated MG). AHP half-decay time was still an accurate predictor of fast and slow motor unit types. Correlations were restored between AHP half-decay time and twitch time-to-peak, and between axonal conduction velocity and twitch time-to-peak, twitch tension, and tetanic tension. We conclude that for self-regenerated MG motoneurons, and MG motoneurons innervating LG, motoneuron properties are little influenced by the particular muscle fibers innervated. A shift towards the longX pattern of relationships between motoneuron electrical properties was evident at ten weeks post-operative.

Although X-soleus was apparently innervated by a full complement of MG motoneuron types, in normal MG proportions, soleus muscle fibers 'resisted' alteration of properties dictated by the motoneuron. This did not result in a mismatch between motoneuron electrical properties and muscle unit contractile properties, as there were compensatory changes in the motoneurons. The overall mean values for motoneuron electrical properties differed significantly between MG motoneurons which innervated soleus (nine months), and those which innervated MG or LG. In all cases, the means differed in the direction of properties of soleus motoneurons. This is also in the direction of normal MG type S motoneurons. The pattern for medX-soleus was similar, although not as developed as at ten months.

The mean values for each of the fast motor unit types (FF, FI, FR) in longX-soleus were typical, in most respects, of those of motoneurons of that same type in normal or self-regenerated MG motoneurons. Thus, those motoneurons able to convert their muscle unit to a fast type recovered motoneuron properties to levels typical of that type in normal MG. For X-soleus type S units, motoneuron electrical properties were generally typical of self-regenerated MG type S motoneurons. There was a tendency for rheobase to be lower and input resistance higher than for self-regenerated motoneurons, thus mean rheobase/input resistance was significantly lower in those MG motoneurons innervating soleus type S muscle units (see also Fig. 7-3). (Interestingly, mean rheobase/input resistance is also lower for normal soleus than for normal MG type S: Table 5-5).

The relationship between AHP half-decay time and axonal conduction velocity was different for MG motoneurons which innervated X-soleus type S muscle units than for self-regenerated MG type S motoneurons. Those cells outside of the normal MG range differed in the direction of the normal relationship for soleus motoneurons. AHP alone was slightly less accurate in differentiating fast and slow motor units (80% vs. nearly 100% in normal and self-regenerated MG), yet motoneuron type was an especially strong predictor of motor unit type in X-soleus (92% agreement). These collective data suggest that, although the relationships between motoneuron electrical properties may be somewhat different for MG motoneurons innervating soleus muscle, there is still a strong relationship between motoneuron type and the contractile properties of the muscle unit. We conclude that since MG motoneurons are

unable to convert many of the soleus muscle fibers' type, the motoneuron's properties become altered to preserve concordance between motoneuron and muscle properties. The particular muscle fibers innervated thus exert a regulatory effect upon the motoneuron.

The strong relationship between motoneuron type and muscle unit type in normal triceps surae (Zengel et al. 1985; CHAPTERS III,V), and self- (CHAPTERS III<IV), and cross-reinnervated triceps surae, suggests that this match-up is important functionally. One can envision both proximate and ultimate advantages to coordinated expression of motoneuron and muscle unit properties. An example of the former would be simplification of neural control of gradation of muscle force achieved by matching the ability of the motoneuron to fire repetitively, (which is thought to be limited by AHP duration; Kernell, 1965; Gustafsson, 1974), and twitch time-to-peak (which is related to the frequency at which muscle unit twitches fuse into tetanic contraction; Henneman, 1980b). An ultimate advantage to precise matching of muscle unit and motoneuron properties is suggested by a recent theory for the cause of muscle wastage in Duschene's muscular dystrophy: Vrbova (1983) hypothesized that the active destruction of dystrophic muscle fibers might be due to a mismatch between motoneuron firing rate, and the ability of the muscle to respond to that frequency of activation.

The MG motoneurons which innervated soleus depart from the trajectory followed by X-LG or self-regenerated MG motoneurons (Fig. 7-5). Comparison of the trajectories for recovery of motoneuron electrical properties during self- and cross-reinnervation indicates that the different values for mean rheobase, input resistance and axonal

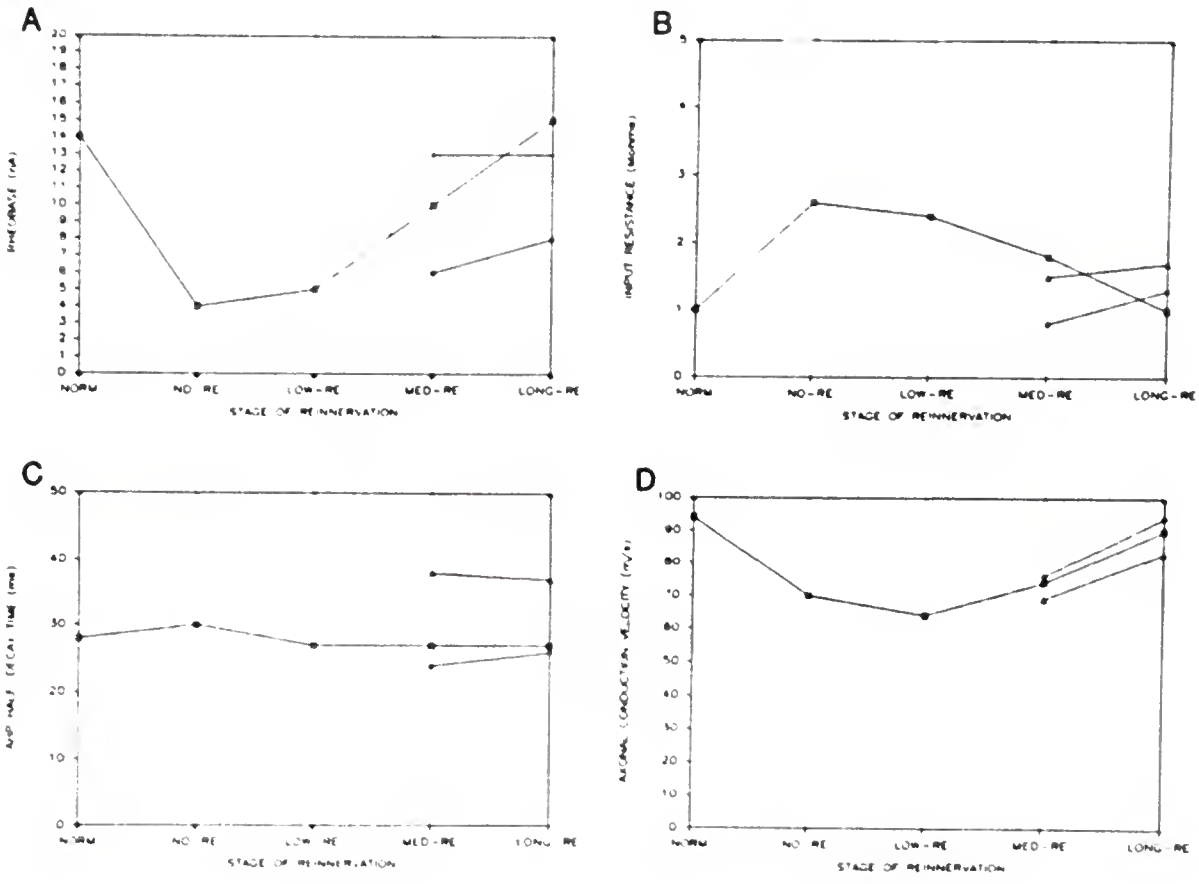

Figure 7-5. Overall mean values for MG motoneuron electrical properties at various times after self-regeneration, or X-regeneration into LG or soleus muscle. (A) rheobase (B) input resistance (C) AHP half-decay time (D) axonal conduction velocity. ▫ = self-regenerated MG. ◇ = X-LG. △=X-soleus.

conduction velocity in X-soleus might reflect incomplete recovery from the axotomized state. For all but axonal conduction velocity, mean values at the medX stage were similar to those at the longX stage. In X-LG, the increased scatter in values for motoneuron electrical properties, and the relationship between rheobase and input resistance (Fig. 7-4), may also reflect incomplete recovery. This suggests that motoneuron electrical properties may recover more slowly (or less completely) when innervating a foreign muscle, even one with a muscle fiber profile and functional role similar to the original muscle.

The AHP half-decay times for MG motoneurons innervating soleus muscle are lengthened, while nerve section alone resulted in no change of mean AHP half-decay time (Fig. 7-5C; see also Kuno *et al.* 1974a; CHAPTER IV; Gustafsson and Pinter, 1984). Thus incomplete recovery could not account for this difference. Based on experiments using a variety of paradigms, Kuno and coworkers (Huiszar *et al.* 1977; Czeh *et al.* 1978; Gallego *et al.* 1978, 1979) hypothesize that AHP duration is regulated by trophic signals from the muscle, which are related to the metabolic state of the muscle (Gallego *et al.* 1979). Our data are consistent with this hypothesis.

Perhaps motoneurons innervating a foreign muscle remain in a 'growth state' (Watson, 1976) and therefore exhibit immature (dedifferentiated) membrane electrical properties. One possibility is that motoneurons receive a trophic signal (molecule) of different quantity or quality from soleus, as compared to the gastrocnemii. Transported to the soma, this signal could then influence the metabolic state, and thus the electrical properties of the motoneuron.

An alternative possibility is that altered synaptic inputs alter motoneuron properties as a function of altered activity. We feel this is unlikely because segmental inputs to motoneurons do not appear to be altered significantly more after cross-reinnervation, than after self-reinnervation (unpublished observations). While monosynaptic Ia EPSPs are smaller than normal after reinnervation (Eccles et al. 1960, 1962b; Gallego et al. 1980; Goldring et al. 1980; Gregory et al. 1982), the magnitude of the changes in amplitude are similar after self- and cross-reinnervation (unpublished observations). More importantly, studies of both group Ia afferent input (Eccles et al. 1962; Mendell and Scott, 1975; Dum et al. 1985a,b), and inputs from motor pattern generators (as represented in electromyographic patterns; Sperry, 1945; Brinkman et al. 1983; Mulkey, 1983; O'Donovan et al. 1985; Luff and Webb, 1982), indicate that these important influences on motoneuron activity are unaltered in pattern following cross-reinnervation. Dorsal rhizotomy has minimal effects on motoneuron AHP duration, axonal conduction velocity, and action potential overshoot (Kuno et al. 1974b). Thus, while muscle nerve activity may be important in regulating motoneuron electrical properties (Czeh et al. 1978; Munson et al. 1985), this effect is likely to be due to activity-related uptake of trophic substance(s) at the neuromuscular junction.

CHAPTER VIII
CONCLUSIONS

This study investigated aspects of the relationships between motoneuron electrical properties and muscle phenotype. The goal was to utilize nerve section and reinnervation of different muscles to determine the extent of motoneuron and motor unit plasticity. This would provide insight into the development and maintenance of the close matching of motoneuron electrical properties and muscle unit contractile properties, found in normal cat MG (c.f. Zengel *et al.* 1985).

The generality of this close match between motoneuron type and muscle unit type was established for normal LG and soleus motor units (CHAPTER 5), as well as confirmed for MG (CHAPTER 3; see also Zengel *et al.* 1985). Following nerve section, and prior to reinnervation of muscle, motoneurons dedifferentiate with respect to motoneuron properties (CHAPTER 4; Kuno *et al.* 1974a; Gustafsson, 1979; Gustafsson and Pinter, 1984). In addition, motoneuron types cannot be recognized at this stage (CHAPTER 4). This indicates that functional connection to muscle is a necessary condition for expression of mature motoneuron electrical properties. Additional evidence in support of this assertion comes from the lack of recovery of electrical properties, of motoneurons in the self- and cross-reinnervation paradigms which fail to reinnervate extrafusal muscle fibers (non-contracts; CHAPTERS 3,4,7).

If functional contact is made between regenerating MG motoneurons, and MG muscle, then the normal proportion of motor unit types is reestablished by nine months post-operative. At the earliest stages of reinnervation, motor unit types cannot be recognized, and motoneuron electrical properties are unchanged from the axotomized state. This suggests that time is required after reinnervation, to re-establish motor unit properties. Recovery of muscle unit contractile properties and motoneuron electrical properties is gradual, with motor unit types recognizable by nine-to-ten weeks. At this time mean values for most motoneuron and muscle unit properties are incompletely recovered, and a larger proportion of fast units are fatigue-resistant. The relationships between motoneuron properties have incompletely shifted toward normal levels by nine-to-ten-weeks post-operative. The re-establishment of motor unit types involves at least some conversion of muscle properties by the motoneuron, as evidenced by muscle fibre type-grouping (Karpati and Engel, 1968). Thus by nine months self-reinnervation, MG motoneuron electrical properties, muscle unit contractile properties, and their relationships have completely recovered (CHAPTER 3). The process of recovery parallels, to some extent, that of normal ontogeny of motor units (CHAPTER 4). The results of this study confirm that the motoneuron plays an important regulatory role in the expression of muscle phenotype.

Functional connection to extrafusal muscle fibers may be a necessary condition for expression of normal, mature motoneuron electrical properties; for long-term self-reinnervation it appears to be a sufficient condition. The question then becomes whether the particular

muscle innervated influences the ultimate expression of motoneuron electrical properties. An important corollary question concerns whether the motoneuron influence on muscle properties is complete, or whether there are limits to this control. To test these hypotheses, we surgically re-routed the MG nerve into the combined LG-soleus nerve. This allowed the above tests, as well as a test of whether reinnervation of foreign muscles was selective, on the basis of motor unit type.

We found that reinnervation of LG and soleus muscles by MG nerve was not selective on the basis of motor unit type (CHAPTER VI). Within MG, LG, and soleus muscles there may, however, be a slight advantage for type S axons, and disadvantage for type FR axons, in capturing and/or maintaining contacts with muscle fibers (CHAPTERS III, IV, VI). The absence of selective reinnervation suggests that with cross-reinnervation, both LG and soleus muscles receive a full complement of MG motoneuron types, in normal MG motor unit type proportions (CHAPTER VI).

LG muscle fibers respond to MG motoneurons in a similar manner to MG muscle fibers. Motor unit types in X-LG are in normal MG proportions, and mean values for motoneuron electrical properties and muscle unit contractile properties recover to self-reinnervated MG levels. The relationships between motoneuron electrical properties, and between motoneuron type and muscle unit type, are of slightly weaker strength, and may be somewhat different in quality, than in normal or self-regenerated MG (CHAPTER VI, VII).

In contrast, soleus muscle fibers 'resist' the influence of MG motoneurons. The X-soleus muscle remains predominately slow, in terms of

motor unit or muscle fiber type proportions (CHAPTER VI). Thus adult soleus muscle lacks the degree of plasticity evident in MG or LG. This incomplete conversion is also true of mean values for, and frequency distributions of, individual muscle unit contractile properties (CHAPTER VI). The classical observation of less complete conversion of slow muscle by a mixed nerve (Buller et. al. 1960) is due to incomplete conversion of individual muscle fibers, as well as the small size of X-soleus fast motor units, and differences in muscle architecture.

This failure of muscle fibers to alter their properties did not result in a mismatch between motoneuron electrical properties and muscle unit contractile properties. Overall mean values for motoneuron parameters were shifted in the direction of type S motoneuron properties. Within both fast and slow motor unit types, motoneuron properties were typical of the same type in normal or self-regenerated MG motoneurons. Motoneuron type was a particularly strong predictor of motor unit type in X-soleus units (CHAPTER VII). The most parsimonous interpretation of these results is that since MG motoneurons were unable to alter soleus muscle fiber properties, the motoneuron properties becamr altered to preserve a strong relationship between motoneuron electrical properties and muscle unit contractile properties. Additional evidence in support of this notion comes from the relationship between AHP half-decay time and axonal conduction velocity for slow motoneurons in X-soleus, which are outside the normal MG range, in the direction of the relationship found in normal soleus motoneurons (CHAPTERS V, VII).

The trajectory of recovery for mean values of MG motoneuron electrical properties during reinnervation, suggests that the altered

values for rheobase, input resistance, and axonal conduction velocity in MG motoneurons which innervated soleus, might be explained by incomplete recovery from the axotomized state. This suggests that muscle plays a permissive role in regulating expression of motoneuron electrical properties. The AHP half-decay times of MG motoneurons which innervated soleus were lengthened, while nerve section alone resulted in no change of mean AHP half-decay time. Alteration of this parameter cannot be explained by incomplete recovery. A retrograde chemical message, which differs in quantity or quality between muscles seems a likely mediating agent for these changes.

This study provides new information regarding the limits of motoneuron influence on muscle phenotype, as well as the documentation of a muscle influence upon expression of motoneuron electrical properties. Such information provides insight into possible mechanisms for ontogenetic or evolutionary specialization of muscle function. Alterations to the motoneuron could be primary, with muscle alterations a secondary consequence, or effects could be direct upon the internal program for muscle fiber development, with motoneuron properties regulated by a feed-back mechanism. This latter course may be represented by cat soleus muscle, a slow muscle specialized (presumably) for maintenance of posture, and slow locomotion (see Burke, 1981). The LG_m compartment of the LG muscle may also represent specialization of the muscle internal developmental program.

A close match between motoneuron and muscle properties would seem to be of advantage for simplifying neural control of muscle, as well as for long-term maintenance of neuromuscular health. Duschenne muscular

dystrophy may result from an imbalance between motoneuron and muscle properties (Vrbova, 1983). The evident plasticity in the adult nervous system would be adaptive for recovery from injury, as well as for maintaining matched motoneuron and muscle properties during seasonal or aging-related alterations in body mass and activity.

LITERATURE CITED

Ariano, M.A., Armstrong, R.B. and Edgerton, V.R. Hindlimb muscle fiber populations of five mammals. J. Histochem. Cytochem. 21: 51-55, 1973.

Bagust, J. Relationship between motor nerve conduction velocity and motor unit contraction characteristics in a slow twitch muscle of the cat. J. Physiol. 231: 87-104, 1974.

Bagust, J. and Lewis, D.M. Isometric contractions of motor units in self-reinnervated fast and slow twitch muscles of the cat. J. Physiol. 237: 91-102, 1974.

Bagust, J., Lewis, D.M. and Luck, J.C. Post-tetanic effects in motor units of fast and slow twitch muscle of the cat. J. Physiol. 237: 115-121, 1974a.

Bagust, J., Lewis, D.M. and Westerman, R.A. Polyneuronal innervation of kitten skeletal muscle. J. Physiol. 229: 241-255, 1973.

Bagust, J., Lewis, D.M. and Westerman, R.A. The properties of motor units in a fast and a slow muscle during post-natal development in the kitten. J. Physiol. 237: 75-90, 1974b.

Bagust, J., Lewis, D.M. and Westerman, R.A. Motor units in cross-reinnervated fast and slow twitch muscles of the cat. J. Physiol. 313: 223-235, 1981.

Baldwin, K.M., Roy, R., Sacks, R.D., Blanco, C. and Edgerton, V.R. Relative independence of metabolic enzymes and neuromuscular activity. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 56: 1602-1607, 1984.

Bardeen, C.R. Development and variation of the nerves and the musculature of the inferior extremity and of the neighboring regions of the trunk in man. Am. J. Anat. 6: 259-390, 1906.

Barrett, E.F., Barrett, J.N. and Crill, W.E. Voltage-sensitive outward currents in cat motoneurones. J. Physiol. 304: 251-276, 1980.

Beery, C.M., Grundfest, H. and Hinsey, J.C. The electrical activity of regenerating nerves in the cat. J. Neurophysiol. 7: 103-116, 1944.

Berthold, C-H., Nilsson, I. and Rydmark, M. Axon diameter and myelin sheath thickness in nerve fibres of the ventral spinal root of the seventh lumbar nerve of the adult and developing cat. J. Anat. 136: 483-508, 1983.

Birks, R., Katz, B. and Miledi, R. Physiological and structural changes at the amphibian myoneural junction, in the course of nerve degeneration. J. Physiol. 150: 145-168, 1960.

Boyd, I.A. and Davey, M.R. Composition of Peripheral Nerves. London: E and S. Livingstone, 1968.

Brinkman, C., Porter, R. and Norman, J. Plasticity of motor behavior in monkeys with crossed forelimb nerves. Science 220: 438-440, 1983.

Brown, G.L. and Von Euler, U.S. The aftereffects of a tetanus on mammalian muscle. J. Physiol. 93: 39-60, 1938.

Brown, M.C. and Ironton, R. Motor neurone sprouting induced by prolonged tetrodotoxin block of nerve action potentials. Nature 265: 459-461, 1977.

Brushart, T.M. and Mesulum, M.-Marsel. Alteration in connections between muscle and anterior horn motoneurons after peripheral nerve repair. Science 208: 603-605, 1980.

Buller, A.J., Eccles, J.C., and Eccles, R.M. Interactions between motoneurons and muscles in respect of the characteristic speeds of their responses. J. Physiol. 150: 399-416, 1960.

Buller, A.J. and Lewis, D.M. Further observations on mammalian cross-reinnervated skeletal muscles. J. Physiol. 150: 417-439, 1965.

Buller, A.J., Mommaerts, W.F.H.M. and Seraydarian, K. Enzymic properties of myosin in fast and slow twitch muscles of the cat following cross-reinnervation. J. Physiol. 205: 581-597, 1969.

Burke, R.E. Motor unit types of cat triceps surae muscle. J. Physiol. 193: 141-160, 1967.

Burke, R.E. The stability of motor unit types in response to altered functional demand: Hypertrophy, atrophy, and reinnervation models. In: Adv. Physiol. Sci., Guba, F., Marechal, G. and Takacs, O., eds. vol. 24, pp 45-56, 1980.

Burke, R.E. Motor units: Anatomy, physiology, and functional organization. In: Handbook of Physiology. The Nervous System. Bethesda, MD: Am. Physiol. Soc., sect. 1, vol. II, part 1, pp 345-422, 1981.

Burke, R.E., Dum, R.P., Fleshman, J.W., Glenn, L.L., Lev-Tov, A., O'Donovan, M.J. and Pinter, M.J. An HRP study of the relation between cell size and motor unit type in cat ankle extensor motoneurones. J. Comp. Neurol. 209: 17-28, 1982.

Burke, R.E., Dum, R.P., O'Donovan, M.J., Toop, J. and Tsairis, P. Properties of soleus muscle and of individual soleus muscle units after cross-reinnervation by FDL motoneurons. Neurosci. Abstr. 5:765, 1979.

Burke, R.E., Levine, D.N., Salcman, M. and Tsairis, P. Motor units in cat soleus muscle: physiological, histochemical and morphological characteristics. J. Physiol. 238: 503-514, 1974.

Burke, R.E., Levine, D.N., Tsairis, P. and Zajac, F.E. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. J. Physiol. 234: 723-748, 1973.

Burke, R.E., Strick, P.L., Kanda, K., Kim, C.C. and Walmsley, B. Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. J. Neurophysiol. 40: 667-680, 1977.

Burke, R.E. and Tsairis, P. Anatomy and innervation ratios in motor units of cat gastrocnemius. J. Physiol. 234: 749-765, 1973.

Cangiano A. and Lutzemberger, L. Partial denervation in inactive muscle affects innervated and denervated fibers equally. Nature 285: 233-235, 1980.

Carmignoto, G., Finesso, M., Siliprandi, R., and Gorio, A. Muscle reinnervation -I. Restoration of transmitter release mechanisms. Neuroscience 8: 393-401, 1983.

Cecchi, G., Lombardi, V. and Menchetti, G. Development of the force-velocity relation and rise of isometric tetanic tension measure the time course of different processes. Pflugers Arch. 401: 396-401, 1984.

Chan, M., Edgerton, V.R., Goslow, G.E., Jr., Kurata, H., Rasmussen, S. and Spector, S.A. Histochemical and physiological properties of cat motor units after self- and cross-reinnervation. J. Physiol. 332: 343-361, 1982.

Clark, D.A. Muscle counts of motor units: a study in innervation ratios. Am. J. Physiol. 96: 296-304, 1931.

Close, R.I. Effects of cross-union of motor nerves to fast and slow skeletal muscles. Nature 206: 831-832, 1965.

Close, R.I. Dynamic properties of mammalian skeletal muscles. Physiol. Rev. 52: 129-197, 1972.

Close, R.I. and Hoh, J.F.Y. Post-tetanic potentiation of twitch contractions of cross-reinnervated rat fast and slow muscles. Nature 221: 179-181, 1969.

Cohen, A. Functional recovery following cross-reinnervation of antagonistic forelimb muscles in rats. Acta. Physiol. Scand. 103: 331-333, 1978.

Cope, T.C, Fournier, M., Bodine, S.C. and Edgerton, V.R. Soleus motor units in adult spinal cats. Neurosci. Abstr. 9: 1224, 1983.

Cragg, G.B. and Thomas, P.K. Changes in conduction velocity and fibre size proximal to peripheral nerve lesions. J. Physiol. 157: 315-327, 1961.

Cragg, G.B. and Thomas, P.K. The conduction velocity of regenerated peripheral nerve fibers. J. Physiol. 171: 164-175, 1964.

Czeh, G., Gallego, R., Kudo, N. and Kuno, M. Evidence for the maintenance of motoneuron properties by muscle activity. J. Physiol. 281: 239-252, 1978.

Davis, L.A., Gordon, T., Hoffer, J.A., Jhamadas, J. and Stein, R.B. Compound action potentials recorded from mammalian peripheral nerves following ligation or suturing. J. Physiol. 285: 543-559, 1978.

Dubowitz, V. Cross-reinnervated mammalian skeletal muscle: histochemical, physiological, and biochemical observations. J. Physiol. 193: 481-496, 1967.

Duchen, L.W. and Strich, S.J. The effects of botulinum toxin on the pattern of innervation in the mouse. Q. J. Exp. Physiol. 53: 84-89, 1968.

Dum, R.P., Burke, R.E., O'Donovan, M.J. and Toop, J. The properties of whole FDL muscle and of individual FDL muscle units after cross-reinnervation by soleus motoneurons in cat. Neurosci. Abstr. 5: 766, 1979.

Dum, R.P., Burke, R.E., O'Donovan, M.J., Toop, J. and Hodgson, J.A. Motor-unit organization in flexor digitorum longus muscle of the cat. J. Neurophysiol. 47: 1108-1125, 1982.

Dum, R.P., O'Donovan, M.J., Toop, J. and Burke, R.E. Cross-reinnervated motor units in cat muscle: 1. Flexor digitorum longus muscle units reinnervated by soleus motoneurons. J. Neurophysiol. In Press, a.

Dum, R.P., O'Donovan, M.J., Toop, J., Tsairis, P., Pinter , M.J. and Burke, R.E. Cross-reinnervated motor units in cat muscle: 2. soleus muscle reinnervated by flexor digitorum longus motoneurons. J. Neurophysiol. In Press, b.

Eccles, J.C., Eccles, R.M. and Kozak, W. Further investigations on the influence of motoneurones on the speed of muscle contraction. J. Physiol. 163: 324-339, 1962.

Eccles, J.C., Eccles, R.M., and Lundberg, A. The action potentials of the alpha motoneurones supplying fast and slow muscles. J. Physiol. 142: 275-291, 1958.

Eccles, J.C., Eccles, R.M. and Lundberg, A. The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurons. J. Physiol. 137: 22-50, 1957.

Eccles, J.C., Eccles, R.M. and Magni, F. Monosynaptic excitatory action on motoneurones regenerated to antagonistic muscles. J. Physiol. 154: 68-88, 1960.

Eccles, J.C., Eccles, R.M., Shealy, C.N. and Willis, W.D. Experiments utilizing monosynaptic excitatory action on motoneurons for testing hypotheses relating to specificity of neuronal connections. J. Neurophysiol. 25: 559-579, 1962.

Eccles, J.C., Libet, B. and Young, R.R. The behaviour of chromatolysed motoneurones studied by intracellular recording. J. Physiol. 143: 11-40, 1958.

Eccles, J.C. and Sherrington, C.S. Numbers and contraction values of individual motor units examined in some muscles of the limb. Proc. Roy. Acad., Ser. B. 106: 326-357, 1930.

Edds, M.V., Jr. Hypertrophy of nerve fibers to functionally overloaded muscles. J. Comp. Neurol. 93: 259-275, 1950.

Edgerton, V.R., Goslow, G.E., Rasmussen, S.A. and Spector, S.A. Is resistance to muscle fatigue controlled by its motor neuron? Nature 285: 589-591, 1980.

Edstrom, L. and Kugelberg, E. Histochemical composition, distribution of fibers, and fatiguability of single motor units. Anterior tibial muscle of the rat. J. Neurol. Neurosurg. Psychiat. 31: 424-433, 1968.

Eerbeek, O., Kernell, D. and Verhey, B.A. Effects of fast and slow patterns of tonic long-term stimulation on contractile properties of fast muscle in the cat. J. Physiol. 352: 73-90, 1984.

Engel, W. Selective and non-selective susceptibility of muscle fiber types. Archs. Neurol. 12: 778-784, 1970.

English, A.W. An electromyographic analysis of compartments in cat lateral gastrocnemius muscle during unrestrained locomotion. J. Neurophysiol. 52: 114-125, 1984.

English, A.W. and Letbetter, W.D. Anatomy and innervation patterns of cat lateral gastrocnemius and plantaris muscles. Am. J. Anat. 164: 67-77, 1982a.

English, A.W. and Letbetter, W.D. A histochemical analysis of identified compartments of cat lateral gastrocnemius muscle. Anat. Rec. 204: 123-130, 1982b.

English, A.W. and Weeks, O.I. Compartmentalization of single motor units in cat lateral gastrocnemius. Exp. Brain Res. 56: 361-368, 1984.

Faber, D.S. Reorganization of neuronal membrane properties following axotomy. Exp. Brain Res. Suppl. 9: 225-239, 1984.

Fleshman, J.W., Munson, J.B., Sypert, G.W. and Friedman, W.A. Rheobase, input resistance, and motor-unit type in medial gastrocnemius motoneurons in the cat. J. Neurophysiol. 46: 1326-1338, 1981.

Frank, E., Jansen, J.K.S., Lomo, T. and Westgaard, R.H. The interaction between foreign and original motor nerves innervating the soleus muscle of rats. J. Physiol. 247: 725-743, 1975.

Gallego, R., Huizar, P., Kudo, N. and Kuno, M. Disparity of motoneurone and muscle differentiation following spinal transection in the kitten. J. Physiol. 281: 253-265, 1978.

Gallego, R., Kuno, M., Nunez, R. and Snider, W.D. Dependence of motoneurone properties on the length of immobilized muscle. J. Physiol. 291: 179-189, 1979a.

Gallego, R., Kuno, M., Nunez, R. and Snider, W.D. Disuse enhances synaptic efficacy in spinal motoneurones. J. Physiol. 291: 191-205, 1979b.

Gallego, R., Kuno, M., Nunez, R. and Snider, W.D. Enhancement of synaptic function in motoneurones during peripheral sensory regeneration. J. Physiol. 306: 205-218, 1980.

Gardiner, P.F., Botterman, B.R., Eldred, E., Simpson, D.R. and Edgerton, V.R. Metabolic and contractile changes in fast and slow muscles of the cat after glucocorticoid-induced atrophy. Exp. Neurol. 62: 241-255, 1978.

Gauthier, F., Burke, R.E., Lowey, S. and Hobbs, A.W. Myosin isozymes in normal and cross-reinnervated cat skeletal muscle fibers. J. Cell Biol. 97: 756-771, 1983.

Gillespie, M.J. and Stein, R.B. The relationship between axon diameter, myelin thickness, and conduction velocity of mammalian peripheral nerves. Brain Res. 259: 41-56, 1982.

Goldring, J.M., Kuno, M., Nunez, R. and Snider, W.D. Reaction of synapses on motoneurones to section and restoration of peripheral connections in the cat. J. Physiol. 309: 185-198, 1980.

Goldring, J.M., Kuno, M., Nunez, R. and Weakly, J.N. Do identical activity patterns in fast and slow motor axons exert the same influence on the twitch time of cat skeletal muscle? J. Physiol. 321: 211-223, 1981.

Gordon, T. Dependence of peripheral nerves on their target organs. In: Somatic and Autonomic Nerve-Muscle Interactions. Burnstock, G., Vrbova, G. and O'Brien, R.A., eds. pp 290-325, 1983.

Gordon, T. and Stein, R.B. Reorganization of motor-unit properties in reinnervated muscles of the cat. J. Neurophysiol. 48: 1175-1190, 1982a.

Gordon, T. and Stein, R.B. Time course and extent of recovery in reinnervated motor units of cat triceps surae muscles. J. Physiol. 323: 307-323, 1982b.

Gregory, J.E., Luff, A.R. and Proske, U. Muscle receptors in the cross-reinnervated soleus muscles in the cat. J. Physiol. 331: 367-383, 1982.

Gustafsson, B. After-hyperpolarization and the control of repetitive firing in spinal neurones of the cat. Acta Physiol. Scand. Suppl. 416: 1-47, 1974.

Gustafsson, B. Changes in motoneurone electrical properties following axotomy. J. Physiol. 293: 197-215, 1979.

Gustafsson, B. and Pinter, M. Effects of axotomy on the distribution of passive electrical properties of cat motoneurones. J. Physiol. 356: 433-442, 1984.

Gustafsson, B. and Pinter, M. Factors determining the variation of the afterhyperpolarization duration in cat lumbar a-motoneurones. Brain Res. 326: 392-395, 1985.

Guth, L. and Samaha, F.J. Procedure for the histochemical demonstration of actomyosin ATPase. Exp. Neurol. 28: 365-367, 1970.

Gutman, E., and Sanders, F.K. Recovery of fiber numbers and diameters in the regeneration of peripheral nerves. J. Physiol. 101: 489-518, 1943.

Hamant, M.F. The motor units of cat lateral gastrocnemius. Unpublished M.S. thesis, Northern Az. Univ. Flagstaff, AZ: 60pp, 1977.

Hammarberg, C. The histochemical appearance of developing muscle fibers in the gastrocnemius, soleus, and anterior tibial muscles of the kitten, as viewed in serial sections stained for lipids and succinate dehydrogenase. Acta. Neurol. Scand. 50: 285-301, 1974.

Hammarberg, C. and Kellerth, J.-O. Studies of some twitch and fatigue properties of different motor unit types in the ankle muscles of the adult cat. Acta Physiol. Scand. 95: 231-242, 1974.

Hammarberg, C. and Kellerth, J.-O. The postnatal development of some twitch and fatigue properties of single motor units in the ankle muscles of the kitten. Acta. Physiol. Scand. 95: 243-257, 1975.

Helwig, J.T. and Council, K.A. SAS User's Guide. Raleigh, NC: Statistical Analysis Systems Institute, 1979.

Henneman, E. Organization of the motoneuron pool: The size principle. In: Medical Physiology, Mountcastle, V.B., ed., vol. one, pp 718-741, 1980a.

Henneman, E. Skeletal muscle: The servant of the nervous system. In: Medical Physiology, Mountcastle, V.B., ed., vol. one, pp 674-702, 1980b.

Henneman, E. and Olson, C.B. Relations between the structure and function in the design of skeletal muscles. J. Neurophysiol. 28: 581-598, 1965.

Hoffer, J.A., Stein, R.B. and Gordon, T. Differential atrophy of sensory and motor nerve fibers following section of cat peripheral nerves. Brain Res. 178: 347-361, 1979.

Hoh, J.F.Y. Neural regulation of muscle regulation. Exp. Neurol. 45: 241-256, 1974.

Hoh, J.F.Y. Selective and non-selective reinnervation of fast-twitch and slow-twitch rat skeletal muscle. J. Physiol. 251: 791-801, 1975.

Hudlicka, O., Tyler, K.R., Srihari, T., Heilig, A. and Pette, D. The effect of different patterns of long-term stimulation on contractile properties and myosin light chains in rabbit fast muscles. Pflugers Arch. 393: 164-170, 1982.

Huiszar, P., Kudo, N., Kuno, M. and Miyata, Y. Reaction of intact spinal motoneurones to partial denervation of the muscle. J. Physiol. 265: 175-191, 1977.

Huiszar, P., Kuno, M. and Miyata, Y. Differentiation of motoneurones and skeletal muscles in kittens. J. Physiol. 252: 465-479, 1975.

Ip, M.C. and Vrbova, G. Reinnervation of the soleus muscle by its own or an alien nerve. Neuroscience 10: 1463-1469, 1983.

Ito, M. and Oshima, T. Electrical behaviour of the motoneurone membrane during intracellularly applied current steps. J. Physiol. 180: 607-635, 1965.

Jami, L. and Petit, J. Correlation between axonal conduction velocity and tetanic tension of motor units in four muscles of the cat hind limb. Brain Res. 96: 114-118, 1975.

Karpati, G. and Engel, W.K. 'Type grouping' in skeletal muscles after experimental reinnervation. Neurology 18: 447-455, 1968.

Kean, C.J.C., Lewis, D.M. and McGarrick, J.D. Dynamic properties of fast and slow twitch muscle of the cat. J. Physiol. 237: 103-113, 1974.

Kellerth, J-O., Mellstrom, A. and Skoglund, S. Postnatal excitability changes of kitten motoneurons. Acta. Physiol. Scand. 83: 31-41, 1971.

Kernell, D. The limits of firing frequency in cat lumbosacral motoneurone possessing different time course of afterhyperpolarization. Acta Physiol. Scand. 65: 87-100, 1965.

Kiraly, J.K. and Krnjevic, K. Some retrograde changes in function of nerves after peripheral section. Q. J. Exp. Physiol. 64: 244-257, 1959.

Kocsis, J.D. and Waxman, S.G. Long-term regenerated nerve fibres retain sensitivity to potassium channel blocking agents. Nature 304: 640-642, 1983.

Kocsis, J.D., Waxman, S.G., Hildebrand, C. and Ruiz, J.A. Regenerating mammalian nerve fibres: changes in action potential waveform and firing characteristics following blockage of potassium conductance. Proc. Roy. Soc. Lond. B 217: 77-87, 1982.

Krnjevic, K., Puil, E. and Werman, R. EGTA and motoneuronal afterpotentials. J. Physiol. 275: 199-223, 1978.

Kugelberg, E., Edstrom, L.P. and Abbruzzese, M. Mapping of motor units in experimentally reinnervated rat muscle. J. Neurol. Neurosurg. Psychiat. 33: 319-329, 1970.

Kuno, M. Excitability following antidromic activation in spinal motoneurones supplying red muscles. J. Physiol. 149: 374-393, 1959.

Kuno, M. A hypothesis for neural control of the speed of muscle contraction in the mammal. Adv. Biophys. 17: 69-95, 1984.

Kuno, M. and Llinas, R. Enhancement of synaptic transmission by dendritic potentials in chromatolysed motoneurones of the cat. J. Physiol. 210: 807-821, 1970.

Kuno, M., Miyata, Y. and Munoz-Martinez E.J. Differential reaction of fast and slow a motoneurones to axotomy. J. Physiol. 240: 725-740, 1974a.

Kuno, M., Miyato, Y. and Munoz-Martinez, E.J. Properties of fast and slow alpha-motoneurones following motor reinnervation. J. Physiol. 242: 273-288, 1974b.

Kuwada, J.Y. and Wine, J. Transient axotomy-induced changes in the membrane properties of crayfish central neurones. J. Physiol. 317: 435-461, 1981.

Lapointe, M.A. and Gardiner, P.F. Effects of tetrodotoxin-induced disuse on properties of developing rat gastrocnemius muscle. Can. J. Physiol. Pharmacol. 62: 1106-1111, 1984.

Letinsky, M.S., Fischbeck, K.H. and McMahan, U.J. Precision of reinnervation of original post synaptic sites in frog muscle after nerve crush. J. Neurocytol. 5: 691-719, 1976.

Lewis, D.M., Bagust, J., Westerman, R.A., Webb, S.N. and Finol, H.J. Axon conduction velocity modified by reinnervation of mammalian muscle. Nature 270: 745-746, 1978.

Lewis, D.M., Rowlerson, A. and Webb, S. Motor units and immunohistochemistry of cat soleus muscle after long periods of cross-reinnervation. J. Physiol. 325: 395-403, 1982.

Lewis, O.J. The phylogeny of the crural and pedal flexor musculature. Proc. Zool. Soc. Lond. 138: 77-109, 1962.

Lieberman, A.R. The axon reaction: A review of the principal features of perikaryal responses to axon injury. Int. Rev. Neurobiol. 14: 50-115, 1971.

Lomo, T. Westgaard, R.H. and Dahl, H.A. Contractile properties of muscle: Control by pattern of muscle activity in the rat. Proc. Roy. Soc. B. 187: 99-103, 1974.

Lomo, T., Westgaard, R.H. and Engebretsen, L. Different stimulation patterns affect contractile properties of denervated rat soleus muscle. In: Plasticity of Muscle. Pette, D., ed., New York: Walter de Gruyter and Co. pp 297-309., 1980.

Lowrie, M.B., and Vrbova, G. Different patterns of recovery of fast and slow muscles following nerve injury in the rat. J. Physiol. 349: 397-410, 1984.

Luff, A.R. Dynamic properties of fast and slow skeletal muscles in the cat and rat following cross reinnervation. J. Physiol. 248: 83-96, 1975.

Luff, A.R. and Webb, S.R. E.m.g. activity in the cross-reinnervated soleus muscle of unrestrained cats. J. Physiol. : 57P-58P, 1982.

Luff, A.R., Proske, U. and Webb, S.N. The transformation of cross-reinnervated slow-twitch muscle after deafferentation in the cat. Exp. Brain Res. 55: 152-157, 1984.

Mayer, R.F., Burke, R.E., Toop, J., Walmsley, B. and Hodgson, J.A. The effect of spinal cord transection on motor units in cat medial gastrocnemius muscles. Muscle and Nerve 7: 23-31, 1984.

McArdle, J.J. Complex endplate potentials at the regenerating neuromuscular junction of the rat. Exp. Neurol. 49: 629-638, 1975.

McMurrich, J.P. The phylogeny of the crural flexors. Am. J. Anat. 4 : 33-76, 1904.

McPhedran, A.M., Wuerker, R.B. and Henneman, E. Properties of motor units in a homogeneous red muscle (soleus) of the cat. J. Neurophysiol. 28: 72-84, 1965.

Mellstrom, A. and Skoglund, S. Quantitative morphological changes in some spinal cord segments during postnatal development. A study in the cat. Acta. Physiol. Scand. Suppl. 331: 1-84, 1969.

Mendell, L.M., Munson, J.B. and Scott, J.G. Alterations of synapses on axotomised motoneurones. J. Physiol. 255: 67-79, 1976.

Mendell, L.M. and Scott, J.G. The effect of peripheral nerve cross-union on connections of single Ia fibers to motoneurons. Exp. Brain Res. 22: 221-234, 1975.

Mendez, J. and Keys, A. Density and composition of mammalian muscle. Metabolism. 9: 184-188, 1960.

Miledi, R. and Stephani, E. Non-selective reinnervation of slow and fast muscle fibres in the rat. Nature 222: 269-271, 1969.

Milner, T.E. and Stein, R.B. The effects of axotomy on the conduction of action potentials in peripheral sensory and motor nerve fibres. J. Neurol. Neurosurg. Psychiat. 44: 485-496, 1981.

Mommaerts, W.F.M., Seraydarian, K., Suh, M., Kean, C.J.R. and Buller, A.J. Conversion of some biochemical properties of mammalian skeletal muscles following cross-reinnervation. Exp. Neurol. 55: 637-653, 1977.

Mosher, C.G., Gerlach, R.L. and Stuart, D.G. Soleus and anterior tibial motor units of the cat. Brain Res. 44: 1-11, 1972.

Mulkey, R.M. Cross reunion of flexor and extensor nerves in the cat hindlimb and its effect on locomotion, reflexes and voluntary movements. Unpublished M.S. Thesis, Northern Az. University, Flagstaff, AZ., pp 49, 1983.

Munson, J.B., Foehring, R.C. and Sypert, G.W. Nerve block with tetrodotoxin mimics axotomy of cat MG motoneurons. Neurosci. Abstr. 11, 1985.

Murphy, R.A. and Beardsley, A.C. Mechanical properties of the cat soleus muscle in situ. Am. J. Physiol. 227: 1008-1013, 1974.

Novikoff, B., Shin, W.Y. and Drucker, J. Mitochondrial localization of oxidative enzymes: Staining results with two tetrazolium salts. J. Biophys. Biochem. Cytol. 9: 47-61, 1961.

Nystrom, B. Postnatal development of motor nerve terminals in "slow-red" and "fast-white" cat muscles. Acta. Neurol. Scand. 44: 363-383, 1968.

O'Donovan, M.J. Developmental regulation of motor function: an uncharted sea. Med. Sci. Sports. Exerc. 17: 35-43, 1985.

O'Donovan, M.J., Pinter, M.J., Dum, R.P. and Burke, R.E. Kinesiological studies of self- and cross-reinnervated FDL and soleus muscles in freely-moving cats. J. Neurophysiol. In Press.

Padykula, H.A., and Herman, E. The specificity of the histochemical method of adenosine triphosphotase. J. Histochem. Cytochem. 3: 170-195, 1955.

Peter, J.B., Barnard, R.J., Edgerton, V.R., Gillespie, C.A. and Stempel, K.E. Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. Biochemistry 11: 2627-2633, 1972.

Peters, S.E. Innervation and compartmentalization of the lateral gastrocnemius/soleus (LG/S) muscle of the opossum. Am. Zool. 24: 86A, 1984.

Peters, S.E., Mulkey, R., Rasmussen, S.A. and Goslow, G.E., Jr. Motor units of the primary ankle extensor muscles of the opossum (Didelphis virginiana): Functional properties and fiber types. J. Morphol. 181: 305-317, 1984.

Pette, D. Activity-induced fast to slow transitions in mammalian muscle. Med. Sci. Sports Exerc. 16: 517-528, 1984.

Pinter, M.J., Curtis, R.L. and Hosko, M.J. Voltage threshold and excitability among variously sized cat hindlimb motoneurons. J. Neurophysiol. 50: 644-657, 1983.

Prewitt, M.A. and Salafsky, B. Effect of cross-reinnervation on biochemical characteristics of skeletal muscles. Am. J. Physiol. 213: 295-300, 1967.

Price, D.L. The influence of the periphery on spinal motor neurons. Ann. N.Y. Acad. Sci. 228: 355-363, 1974.

Purves, D. Functional and structural change in mammalian sympathetic neurons following colchicine application to post-ganglionic nerves. J. Physiol. 259:159-178, 1976.

Rasmussen, S., Chan, A.K. and Goslow, G.E. The cat step cycle: electromyographic patterns for hindlimb muscles during posture and unrestrained locomotion. J. Morpol. 155: 253-270, 1977.

Ridge, R.M.A.P. The differentiation of conduction velocities of slow twitch and fast twitch muscle motor innervations in kittens and cats. Quart. J. Exp. Physiol. 52: 293-304, 1967.

Ritchie, J.M. Sodium and potassium channels in regenerating and developing mammalian myelinated nerves. Proc. Roy. Soc. Lond. B 215: 273-287, 1982.

Romanul, F.C.A. and Van Der Meulen, J.P. Slow and fast muscles after cross reinnervation. Enzymatic and physiological changes. Arch. Neurol. 17: 387-402, 1967.

Rosenfalck, P. and Buchthal, F. On the concept of the motor subunit. Int. J. Neurosci. 1: 27-37, 1970.

Rothshenker, S. and McMahan, U.J. Altered patterns of innervation in frog muscle after denervation. J. Neurocytol. 5: 719-730, 1976.

Rushmer, D.S., MacPherson, J.M., Dunbar, D.C. and Russell, C.J. Responses of lateral gastrocnemius innervation compartments and soleus to paired perturbations of posture. Neurosci. Abstr. 9: 63, 1984.

Russell, C.J., Dunbar, D.B., Rushmer, D.S., MacPherson, J.M. and Phillips, J.O. Differential activity of innervation subcompartments of cat lateral gastrocnemius during natural movements. Neurosci. Abstr. 8: 948, 1982.

Sacks, R.D. and Roy, R.R. Architecture of the hind limb muscles of cats: functional significance. J. Morph. 173: 185-195, 1982.

Salmons, S. and Henriksson, J. The adaptive response of skeletal muscle to increased use. Muscle and Nerve 4: 94-105, 1981.

Salmons, S. and Vrbova, G. The influence of activity on some contractile characteristics of mammalian fast and slow muscles. J. Physiol. 210: 535-549, 1969.

Saltin, B. and Gollnick, P.D. Skeletal muscle adaptability: significance for metabolism and performance. Handbook of Physiology Peachey, L.D., Adrian, R.H. and Geiger, S.R., eds., Bethesda: Am. Physiol. Soc., Sect. 10: Skeletal Muscle, pp 555-631, 1983.

Sanders, F.K. and Young, J.Z. The influence of peripheral connections on the diameter of regenerating nerve fibers. J. Exp. Biol. 22: 203-212, 1946.

Sanes, J. Role of extracellular matrix in neural development. Ann. Rev. Physiol. 45: 581-600, 1983.

Sato. M., Mizuno, N. and Konishi, A. Postnatal differentiation of cell body volumes of spinal motononeurons innervating slow-twitch and fast-twitch muscles. J. Comp. Neurol. 175: 27-36, 1977.

Schiebel, M.E. and Scheibel, A.B. Developmental relationship between spinal motoneuron dendrite bundles and patterned activity in the hind limb of cats. Exp. Neurol. 29: 328-335, 1970.

Slack, J.R., Hopkins, W.G. and Pockett, S. Evidence for a motor nerve growth factor. Muscle and Nerve 6: 243-252, 1983.

Smith, D. Miniature stimulator for chronic animals. Pflugers Arch. 376: 93-95, 1978.

Smith, J.L., Edgerton, V.R., Betts, B. and Collatos, T.C. EMG of slow and fast ankle extensors of cat during posture, locomotion, and jumping. J. Neurophysiol. 40: 503-513, 1977.

Spector, S.A. Regulation of muscle size by activity and non-activity-related factors. Neurosci. Abstr. 10: 1060 , 1984.

Spector, S.A., Gardiner, P.F., Zernicke, R.F., Roy, R.R. and Edgerton, V.R. Muscle architecture and the force velocity characteristics of the cat soleus and medial gastrocnemius: Implications for motor control. J. Neurophysiol. 44: 951-960, 1980.

Sperry, R.W. The problem of central nervous reorganization after nerve regeneration and muscle transposition. Quart. Rev. Biol. 20: 311-369, 1945.

Stuart, D.G. and Enoka, R.M. Motoneurons, motor units, and the size principle. In: The Clinical Neurosciences, Grossman, R.J. and Willis, W.D. Jr., eds., Sect. 5- Neurobiology, pp V471-V517 Churchill Livingstone, 1983.

Sypert, G.W. and Munson, J.B. Basis of segmental motor control: motoneuron size or motor unit type? Neurosurgery 8: 608-621, 1981.

Thompson, W.J. Sutton, L.A. and Riley, D.A. Fibre type composition of single motor units during synapse elimination in neonatal rat soleus muscle. Nature 309: 709-711, 1984.

Tomanek, R.J. and Tipton, C.M. Influence of exercise and tenectomy on the morphology of a muscle nerve. Anat. Rec. 159: 105-114, 1967.

Vanden-Noven, S., Hamm, T.M. and Stuart, D.G. Testing for reflex partitioning in the motor nucleus of the cat lateral gastrocnemius muscle. Neurosci. Abstr. 9: 528, 1983.

Vrbova, G. Duchenne dystrophy viewed as a disturbance of nerve-muscle interactions. Muscle and Nerve 6: 671-675, 1983.

Vrbova, G., Gordon, T. and Jones, R. Nerve-Muscle Interaction. London: Chapman and Hall, 1978.

Walsh, J.V. Jr., Burke, R.E., Rymer, W.Z. and Tsairis, P. Effect of compensatory hypertrophy studied in individual motor units in medial gastrocnemius muscle of the cat. J. Neurophysiol. 41: 496-508, 1978.

Watson, W.E. The response of motor neurones to intramuscular injection of botulinum toxin. J. Physiol. 202: 611-630, 1969.

Watson, W.E. Cell Biology of Brain. London: Chapman and Hall, 1976.

Wattenberg, L.W. and Leong, J.L. Effect of coenzyme Q10 and menadione on succinic dehydrogenase activity as measured by tetrazolium salt reduction. J. Histochem. Cytochem. 8: 296-303, 1960.

Weeks, O.I., and English, A.W. Distribution of cell sizes in the cat lateral gastrocnemius motor nucleus. Anat. Rec. 205: 212a-213a, 1983.

Weeks, O.I. and English, A.W. Compartmentalization of cat lateral gastrocnemius motor nucleus. J. Comp. Neurol. 235: 255-267, 1985.

Yahr, M.D., Herz, E., Moldaver, J., and Grundfest, H. Electromyographic patterns in reinnervated muscle. Archs. Neurol. Psychiat., Chicago 63: 728-738, 1950.

Young, B.L., Begovac, P., Stuart, D.G. and Goslow, G.E. Jr. An effective sleeving technique in nerve repair. J. Neurosci. Methods 10: 51-58, 1984.

Zajac, F.E. and Faden, J.S. Relationship among recruitment order, axonal conduction velocity, and muscle-unit properties of type-identified motor units in cat plantaris muscle. J. Neurophysiol. 53: 1303-1322, 1985.

Zengel, J.E., Reid, S.A., Sypert, G.W. and Munson, J.B. Presynaptic inhibition, epsp amplitude, and motor-unit type in triceps surae motoneurons in the cat. J. Neurophysiol. 49: 922-931, 1983.

Zengel, J.E., Reid, S.A., Sypert, G.W., and Munson, J.B. Membrane electrical properties and prediction of motor unit type of medial gastrocnemius motoneurons in the cat. J. Neurophysiol. 53: 1323-1344, 1985.

BIOGRAPHICAL SKETCH

I was born July 3, 1955, in St. Albans, N.Y. I attended primary schools in Connecticut, New Jersey and Vermont. I graduated from Champlain Valley Union High School, in Vermont, with an interest in wildlife biology. I majored in biology at the University of Vermont, graduating magna cum laude, in 1977. Between my undergraduate and graduate training, I spent a year working construction and traveling and collecting plants in South America. While at Vermont, I became interested in functional morphology. I enrolled in an M.Sc. program at Northern Arizona University to study with Dr. G.E. Goslow. While living in Flagstaff, I met and married my wife, Deb. I obtained my master's degree in biology in 1980, then entered a Ph.D. program in zoology at Washington State University. After a year at Washington State, I made a major career change, and moved to the University of Florida. While in Gainesville, I have had the great fortune to work with Drs. John Munson and George Sypert. Gainesville is also the birthplace of my daughter, Christen Noelle.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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